DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NEW HETEROCYCLIC DERIVATIVES AS ANTI-TUBERCULAR AGENTS

A dissertation submitted to

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In partial fulfilment of the requirements for the award of degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL CHEMISTRY

Submitted by 261415710

Under the guidance of

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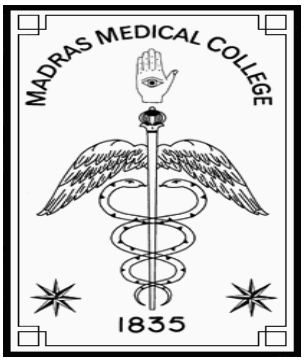

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CERTIFICATE

This is to certify that the dissertation entitled **"DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL HETEROCYCLIC DERIVATIVES AS ANTI- TUBERCULAR AGENTS"** submitted by the candidate bearing the register No:**261415710** in partial fulfillment of the requirements for the award of degree of **MASTER OF PHARMACY** in **PHARMACEUTICAL CHEMISTRY** by the Tamilnadu **Dr. M.G.R Medical University** is a bonafide work done by him during the academic year 2015-2016 at the **Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai- 600 003.**

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Professor and Head,
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Dr. A .JERAD SURESH,
Principal,
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CONTENTS

LIST OF ABBREVIATIONS

WHO	World Health Organisation
Mtb	Mycobacterium Tuberculosis
MDR-TB	Multi Drug Ressistant Tuberculosis
XDR-TB	Extensively Drug Ressistant Tuberculosis
HIV	Human Immuno Deficiency Syndrome
AIDS	Acquired Immuno Deficiency Syndrome
NSAID	Non Steriodal Anti Inflammatory Drugs
GLIDE	Grid Based Ligand Docking Energetics
QSAR	Quantitative Structure Activity Relationship
NMR	Nuclear Magnetic Resonance Spectroscopy
3D	3 Dimentional
Pdb	Protein Data Bank
ADME	Absorption Distribution Metabolism and Excretion
BBB	Blood Brain Barrier
PSA	Polar Surface Area
OSIRIS	Optical, Spectroscopic and Infra red Remote Imaging System
IR	Infra-red Spectroscopy
KBr	Potassium bromide
NMR	Nuclear Magnetic Resonance Spectroscopy
DMSO	Di Methyl Sulfoxide
GC-MS	Gass Chromatography and Mass Spectroscopy
MABA	Microplate Alamar Blue Assay
MIC	Minimum Inhibitory Concentration
TB	Tuberculosis

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“Gratitude makes sense of our past, brings peace for today and creates a vision for tomorrow”.

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1. INTRODUCTION

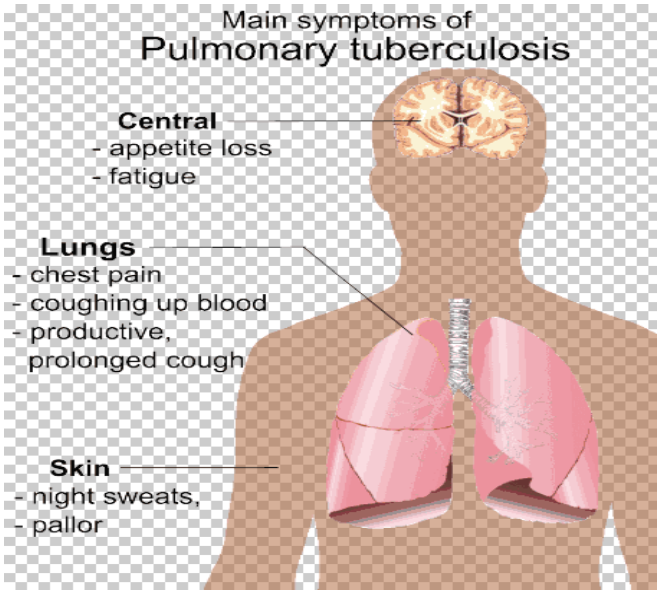
Tuberculosis is an infectious disease usually caused by the bacteria Mycobacterium tuberculosis. Tuberculosis may infect any part of the body, but most commonly occur in the lungs (known as pulmonary tuberculosis). Extrapulmonary TB occurs when tuberculosis develops outside of the lungs. Extrapulmonary TB may co exist with pulmonary TB. The classic symptoms of active TB are chronic cough with blood-tinged sputum, fever, night sweats and weight loss. Tuberculosis is spread through the air when people who have active TB in their lungs cough, spit, speak or sneeze.[1-2] Active infection occurs more often in people with HIV/AIDS and in those who smoke.

One third of the world population is thought to be infected with TB .[1] New infection occur in about 1% of the population each year.[3] In 2014 , there where 9.6 million case of active TB which resulted in 1.5 million deaths .More than 95% of deaths occurred in developing countries [1]. About 80% of the people in many Asian and African countries test positive while 5-10 % of people in the United Status population tests positive by the tuberculin test .Tuberculosis has been present in humans since ancient times .[4]

GENERAL SIGNS AND SYMPTOMS

General signs and symptoms include fever, chills, night sweats, loss of appetite, weight loss, and fatigue. Significant nail clubbing may also occur. [5]

Figure. 1 [6]



BACKROUND

Tuberculosis or TB is the most common infectious disease. In the past Tuberculosis also called as phthisis or phthisis pulmonalis. TB is second only to HIV/AIDS as the greatest killer worldwide due to the single infectious agent.[7]

In addition, the prevalence of drug-resistant TB is also increasing worldwide. Co-infection with HIV has been an important factor in the emergence in the spread of resistance.[8] New TB treatments are being developed and new vaccines are currently under investigation[9]. TB is a major global health threat, and we must improve the existing treatment regimen to control the spread of TB.

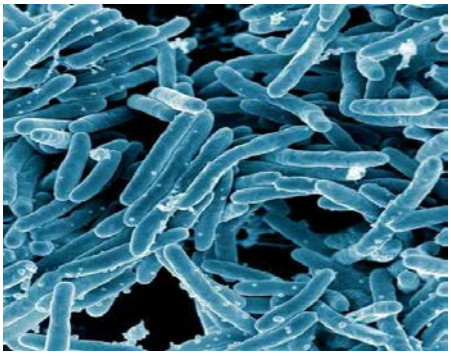
HISTORY

M. Tuberculosis, then known as the "Tubercle Bacillus", was first described on 24 March 1882 by Robert Koch, WHO subsequently received the Nobel Prize in physiology or medicine for this discovery in 1905; The Bacterium is also known" Koch's Bacillus".[10] Tuberculosis has existed throughout history, but the name has changed frequently over time. In 1720, though, the History of Tuberculosis started to take shape into what is known of it today, as the physician Benjamin Marten described in his *A Theory of Consumption*, Tuberculosis may be caused by small living creatures transmitted through the air to other patients. [11]

MYCOBACTERIA

The main cause of TB is Mycobacterium tuberculosis which is a rod shaped, small, aerobic, non motile bacillus. The high lipid content of this pathogen accounts for many of its unique clinical characteristics. It divides every 16 to 20 hours .Which is an extremely slow rate compared with other bacteria. Mycobacteria have an outer membrane lipid bilayer. If a gram stain is performed, MTB either stains very weekly gram – positive or does not retain dye as a result of the high lipid and mycolic acid content of its cell wall [12]. In nature the bacterium can grow only within the cells of a host organism, but *M.tuberculosis* can be cultured in the laboratory. Since MTB retains certain stains even after being treated with acidic solution, it is classified as an acid-fast bacillus. [13]

`Figure. 2 [14]



SCIENTIFIC CLASSIFICATION

Kingdom	:	Bacteria
Phylum	:	Actinobacteria
Class	:	Actinobacteria
Class	:	Actinomycetalus
Sub Order	:	Corynebacterineae
Family	:	Mycobacteriaceae
Genus	:	Mycobacterium
Species	:	Mycobacterium tuberculosis [15]

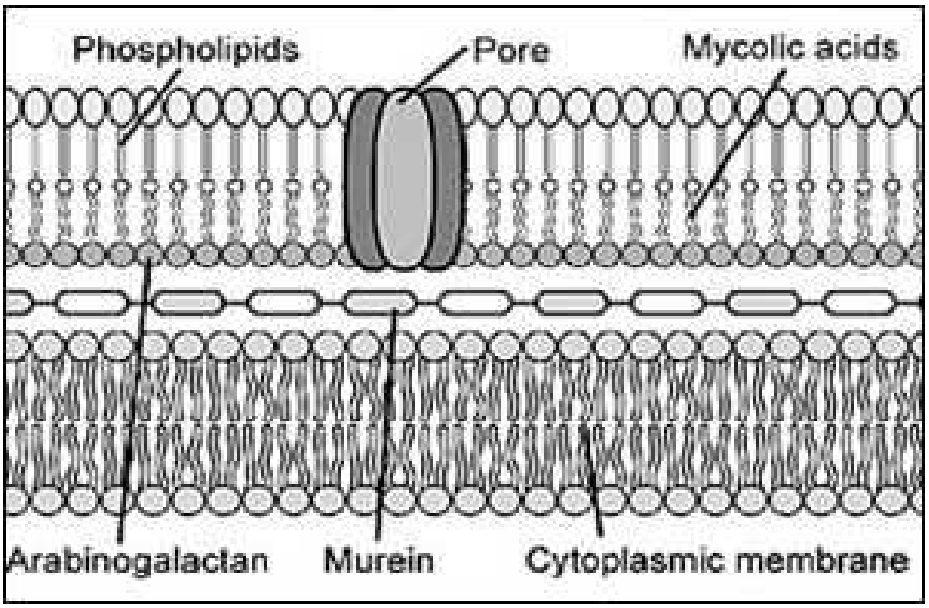
CELL WALL

The well-developed cell wall contains a considerable amount of a fatty acids, mycolicacid which is covalently attached to the underlying peptidoglycon-bound polysaccharide arabinogalactan , providing an extra ordinary lipid barrier .This barrier is responsible for many of the medically challenging physiological characteristics of tuberculosis. The composition and quality of the cell wall components affect the bacteria's virulence and growth rate.

The peptidoglycan polymer confers cell wall rigidity and just external to the bacterial cell membrane another contributer to the permeability barrier of mycobacteria. The peptidoglycan polymer confers cell wall rigidity.

Another important component of the cell wall is lipoarabinomannan, a carbohydrade structural antigen on the organism that is immunogenic and facilitates the survival of mycobacteria within macrophages. The cell wall is key to the survival of mycobacteria and a more complete understanding of the biosynthetic pathways and gene functions and the development of antibiotics to prevent formation of the cell wall are areas of great interest.[16]

Figure. 3[17]



DRUG RESISTANT TB

Drug-resistant TB disease can develop in two different ways, called primary and secondary resistance. Primary resistance occurs in persons who are initially exposed to and infected with resistant organisms .Secondary resistance, or acquired resistance, develops during TB therapy, either because the patient was treated with an inadequate

regimen or did not take the prescribed regimen appropriately, or because of other conditions such as drug malabsorption or drug-drug interactions that led to low serum levels.

MDR TB is caused by organisms resistant to both isoniazid and rifampicin, which are the two most effective anti-TB drugs .These drugs are considered first-line drugs and are used to treat most persons with TB disease.

XDR TB is relatively rare type of drug- resistant TB .XDR TB is resistant to isoniazid and rifampicin ,plus any fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, orcapreomycin).Because XDR TB disease is resistant to first-line and second-line drugs ,patients are left with treatment options that are more toxic ,more expensive, and much less effective.

THE NEED FOR NOVEL TUBERCULOSIS DRUGS

1. To improve current treatment by shortening the total duration of treatment

2. To improve the treatment of MDR-TB[18]

3. To provide for more effective treatment of latent tuberculosis infection [(16)]

4. New drugs to improve current drugs that facilitate compliance by providing for less intensive supervision are also of great interest

5. Discovery of a compound that would reduce both the total length of treatment and the frequency of drug administration

6. MDR TB must be treated with a combination of "second line" drugs which are not only more expensive but also much more toxic and less effective than the drugs used in standard therapy.[19]

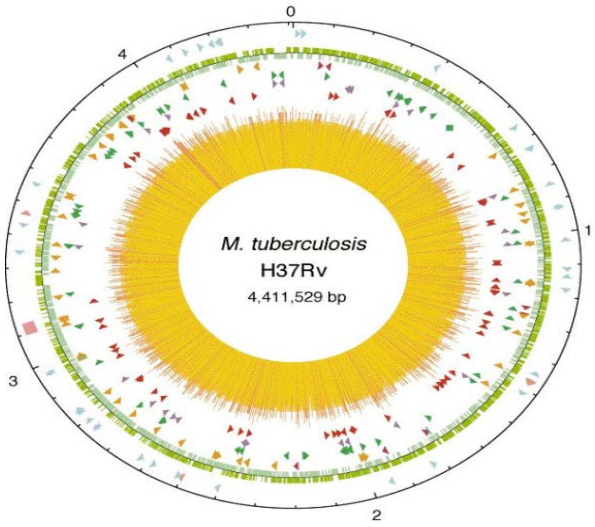
GENOME

The Mycobacterium Tuberculosis genome encodes about 190 transcriptional regulators, including is sigma factors, two-component system and more than 140 transcription regulators. Several regulators have been found to respond to environmental distress, such as extreme cold or heat, iron starvation, and oxidative stress. To survive in these harsh conditions for a prolonged period in the host, Mycobacterium tuberculosis had learned to adapt to the environment by allowing an inhibiting transcription according to it's surrounding. [20]

GENOME STRUCTURE

Mycobacterium tuberculosis has circular chromosomes of about 4,200,000 nucleotides long .The genome of M.tuberculosis was studied generally using the strain M.tuberculosis H37Rv. The genome contains about 4000 genes. Genes that code for lipid metabolism are very important for the bacterial genome, and 8% of the genome is involved in this activity.[21] Plasmid in M.tuberculosis is important in transferring virulence because genes on the plasmids are more easily transferred than genes located on the chromosomes. One such 18kb plasmid in the M.tuberculosis H37Rv strain was proven to conduct gene transfers. [22]

Figure. 4 [22] The chromosome of M.Tuberculosis H37Rv and the gene synthesise mycolic acids.



CHOLESTROL METABOLISM

Cholesterol metabolism has been studied extensively because of its possible therapeutic application in TB infections. It has been shown numerous times that TB requires cholesterol for virulence in vivo, because Mycobacterium Tuberculosis (Mtb); the causative agent, utilizes cholesterol as a source of carbon, energy, and steroid-derived metabolites throughout the course of infection. [23]

ROLE OF CHOLESTROL IN TB INFECTION

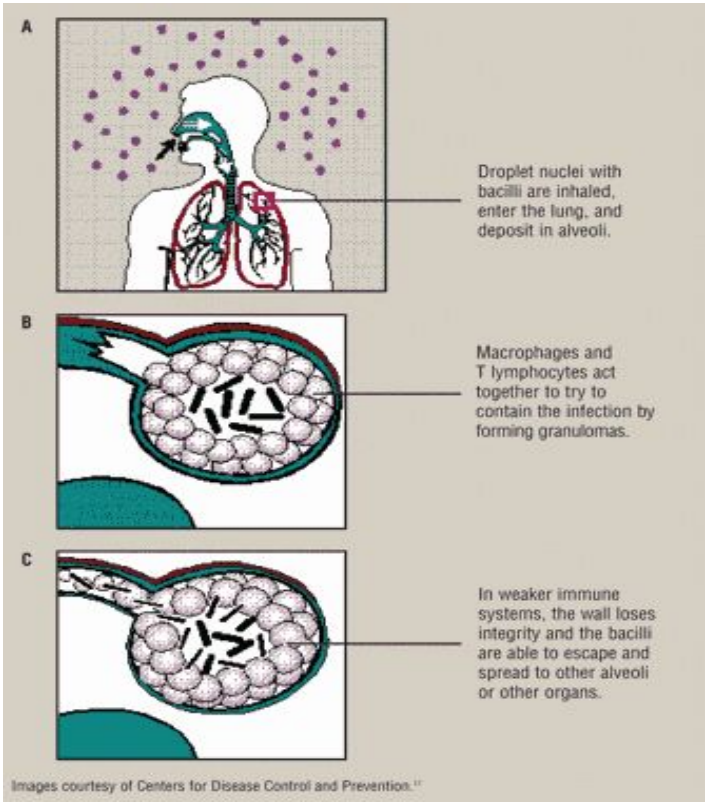
Tuberculosis infection has unique virulence factors compared to most pathogens. The infect host cell and persist inside phagosome, where there are limited nutrients. Tuberculosis has unique ability to utilize cholesterol, which is a common component of human cell membrane, plays a role in its persistence, because the cholesterol catabolism pathway requires a large number of oxygen, that TB infect the lungs where oxygen concentrations are highest.[24]

PATHOPHYSIOLOGY OF TUBERCULOSIS

Mycobacterium tuberculosis requires high levels of oxygen to grow. Primarily a pathogen of the mammalian respiratory system, it infect the lungs .The most frequently used diagnostic methods for TB are the ***tuberculin test, acid- fast stain, and chest radiographs***.[25]

M.tuberculosis is divides every 15-20 hours, which is extremely slow, compared to other bacteria. It is a small bacillus that can withstand weak disinfectants and can survive in a dry state for weeks. Its unusual cell wall, rich in lipids (e.g. mycolic acids).[26] Humans are the only known reservoirs of Mycobacterium tuberculosis. When in the lungs, mycobacterium tuberculosis is taken up by alveolar macrophages, but they are unable to digest and eradicate the bacterium. It cell wall prevents the fusion of the phagosome with theylosome, which contains host of anti- mycobacterial factors. [27]

Figure. 5 [28] Pathophysiology of tuberculosis



(A) inhalation of bacilli, (B) containment in the granuloma ,(C) Breakdown of the granuloma in less immunocompetent individuals .

Need for new anti-TB drugs

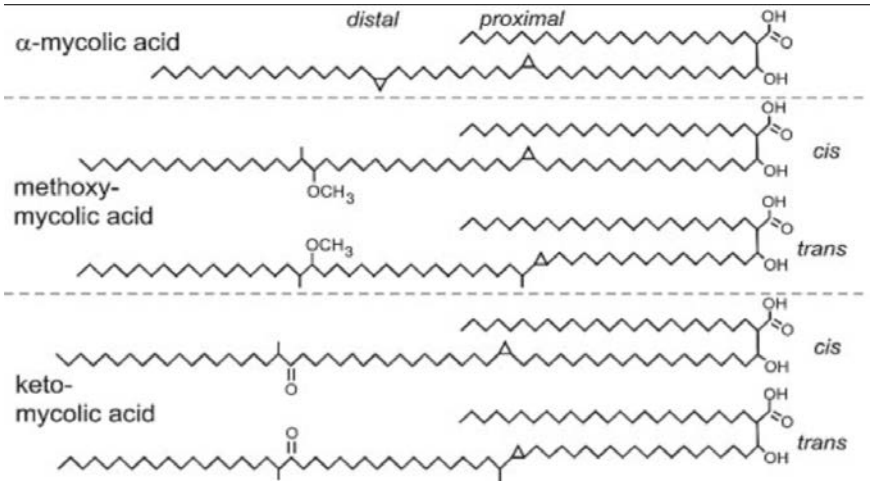
- The recent rise in TB cases and especially the increase of drug resistant mycobacteria indicate an urgent need to develop new anti-TB drugs.
- The long duration of TB therapy is a consequence of persistent mycobacterium tuberculosis, not effectively killed by current anti-TB agents. [29]
- Recent advances in the knowledge of the biology of the organism and the availability of the genome sequence give an opportunity to explore a wide range of novel targets for drug design.

- It is expected that the application of functional genomic tools, such as structure based drug design and combinatorial chemistry will lead to the development of new drugs that are active against drug resistant TB. [30]
- There is a need to design new drugs that are more active against slowly growing and non growing persistent bacilli to meet the population at risk of developing active disease through reactivation.

ENZYME PROFILE

Mycolic acids, a homologous series of C60-C90 long-chain alpha-alkyl- and beta hydroxyl fatty acids, represent essential components of the mycobacterial cell wall. They are important for mycobacterial growth, survival, and pathogenicity. They are found as esters of anarabinogalactan as well as free lipids in the form trialosedimycolate (TDM).Arabinogalactanmycolate is covalently linked to the cell wall peptidoglycan via a phosphodiester bond located on the inner leaflet of the outer membrane. Both arabinogalactan and TDM provide a protective thick cell wall and protect the tubercle bacillus from antibiotics and host‘s immune system.TDM also inhibits phagolysosome fusion and is often considered to be an indicator of virulent strains.

Figure. 6[(31)]



Three distinct structural classes of mycolic acids namely alpha-(more than 70percent), methoxy-and keto-mycolic acids (10-15%) are found in this bacillus. Methoxy-mycolic acids, which contain several methoxy groups, comprise between 10 to

15% of the mycolic acids in the organism. The alpha-mycolic acid is a cis, cis-dicyclopropyl fatty acid. Both methoxy-and keto- mycolic acids have either cis- or trans-cyclopropane rings. Cyclopropane rings in mycolic acids protect the bacillus from oxidative stress.

Several front-line drugs used for treating tuberculosis inhibit mycolic acid synthesis. Understanding the pathway of mycolate biosynthesis and the underlying molecular mechanisms of the disease tuberculosis a well as the identification of new antituberculosis drug targets is important. InhA(ECn1.3.1.9,enoyl-[acyl-carrier-protein]reductase), involved in mycolic acid synthesis ,is a target of front-line anti-tubercular drugs, such as isoniazid and ethionamide. Enzymes needed for biosynthesis of mycolic acids, such as methoxy mycolic acid synthase2, cyclopropane mycolic acid synthase 2, methyl transferase (PcaA),beta-ketoacyl-acyl carrier protein synthase (KasAB and FabH),acyl-AMP ligase (Fad32) and polyketide synthase(Psk13) are promising drug targets for new anti-TB agents.[31]

The presence of mycolic acids gives M.tuberculosis many characteristics that defy medical treatment.Theylend the organism increased resistance to chemical damage and dehydration, and prevent the effective activity of hydrophobic antibiotics .In addition, the mycolic acids allow the bacterium to grow readily inside macrophages, effectively hiding it from the host's immune system. Mycolate bio- synthesis is crucial for survival and pathogenesis of M. Tuberculosis.[32-33]

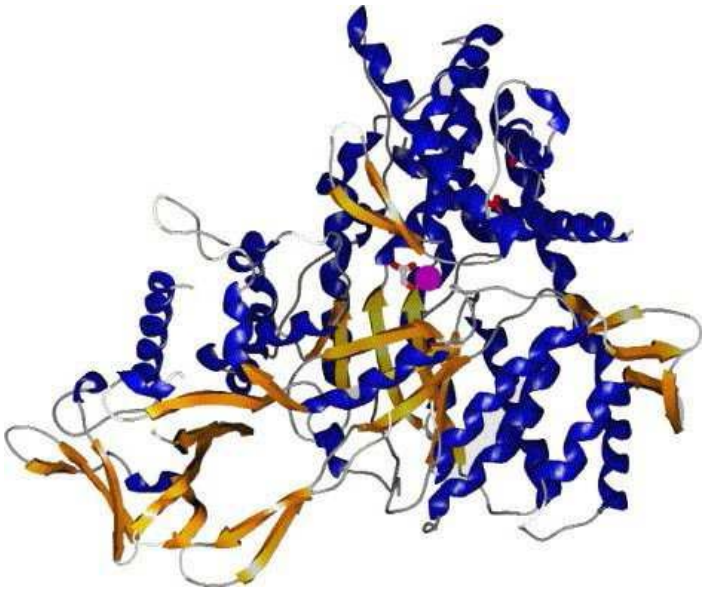
Five distinct stages are involved in biosynthesis of mycolic acid these were summarized as follows

- ✓ Synthesis of the C26 saturated straight chain fatty acids by the enzyme fatty

acid synthase -I (FAS-I) to provide the α-alkyl branch of the mycolic acids.

- ✓ Synthesis of the C56 fatty acids by FAS-II providing the meromycolate backbone.
- ✓ Introduction of functional groups to the meromycolate chain by numerous Cyclopropane synthases.
- ✓ Condensation reaction catalysed by the polyketide synthase Pks13 between the α-branch and the meromycolate chain before a final reduction by the enzyme Corynebacterineae mycolate reductase A (CmrA) to generate the mycolic acid; and Transfer of mycolic acids to arabinogalactan and other acceptors such as trihalose via the antigen 85 complex.
- ✓ The fatty acid synthase-I and fatty acid synthase-II pathways producing mycolic acids are linked by beta-ketoacyl-(acyl-carrier-protein) synthase III enzyme, often designated as mtFabH. Novel inhibitors of this enzyme could potentially be used as therapeutic agents.[(34)]
- ✓ MmA2 is required for introduction of the distal cyclopropane ring in the formation of meroacids. Analysis of a mmA2 deletion mutant of Tuberculosis revealed that mycolic acid lacks a distal cyclopropane group and instead contains a cis unsaturation. Thus, mmA2 is required for the distal cyclopropane modification of mycolic acid. [(35)]

Figure .7 [33] Methoxy Mycolic Acid Synthase 2



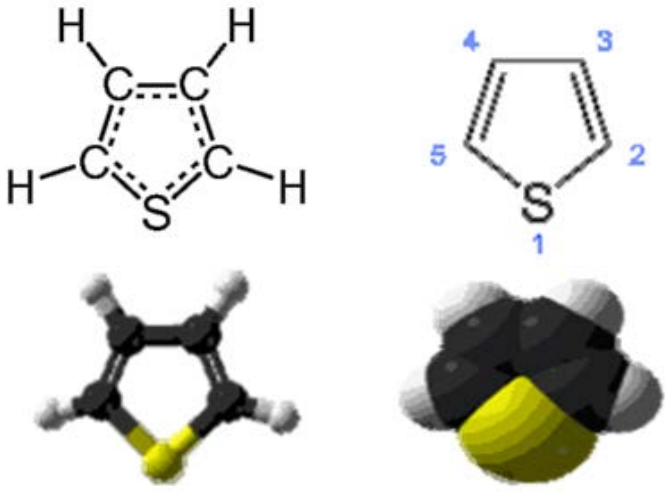
- **ENZYME NAME : Methoxy mycolic acidsynthase2**
- **GENE NAME : mmA2, mmaA2, RV0644C**
- **CLASSIFICATION : Transferase**
- **STRUCTURE WEIGHT : 33493.47**
- **POLYMER : 1**
- **TYPE : Protein**
- **CHAINS : A**
- **ORGA\NISM : Mycobacterium tuberculosis**
- **PROTEOME : Chromosome**
- **FUNCTIONAL CATEGORY : Lipid metabolism [35]**

BASIC NUCLEUS PROFILE

THIOPHENE

Tiophene, a heterocyclic nucleus has attracted a wide attention of the chemist in search for the new therapeutic molecules. Thiophene, also called as thiofuran, is a heterocyclic compound with the formula C4H4S. It consists of a flat five membered ring, it is aromatic as indicated by its extensive substitution reactions. Related to thiophene are benzothiophene, and dibenzothiophene, containing the thiophene ring fused with one and two benzene rings, respectively [(36)].

Figure. 8



IUPAC NAME

Thiophene

Other names

Thiofuran

Thiacyclopentadiene

Thiole

PROPERTIES

Chemical formula : C_4H_4S

Molar mass : 84.14 g/mol

Appearance : colorless liquid

Density : 1.051g/ml

Melting point : - 38c

Boiling point : 84c

Refractive index : 1.5287[36]

USES

Thiophenes are important heterocyclic compounds that are widely used as building blocks in many agrochemicals and pharmaceuticals.The benzene ring of a biologically active compound may often be replaced by a thiophene without loss of activity. This is seen in examples such as the NSAID Iornoxicam, the thiphene analog of piroxicam. [37]

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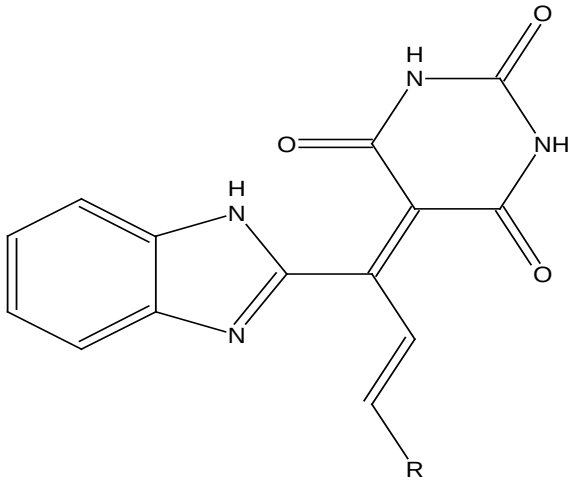
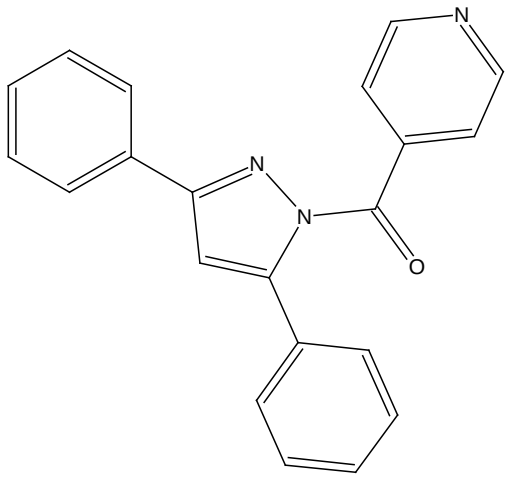
Literature review on chalcones

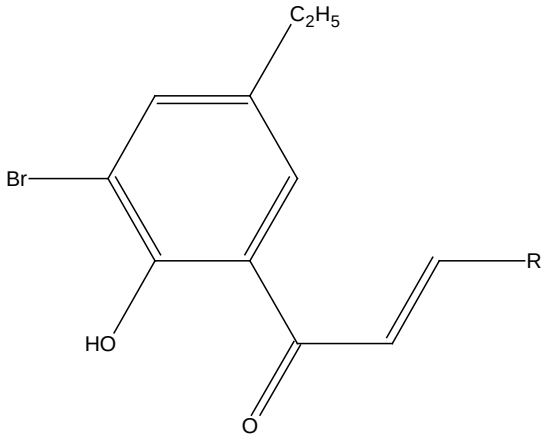
23. Bijo Mathew A J et.al.,(2012)[(53)] The benzimidazole nucleus exhibits wide range of biological activities. The aim of our research was incorporate a barbitone pharmacophore to the benzimidazole chalcones by means of a C=C bond and improve its bioactive nature.The final compound were screened for antimicrobial studies.All the compounds showed a good activity towards Gram positive bacteria and less activity towards Gram negative bacteria.The present study described the synthesis of novel derivatives of 5-[(2E)-1-(1H-benzimidazole-2-yl)-3-substituted phenylprop-2-en-1-ylidene] pyrimidine-2,4,6(1H,3H,5H)-trione by bothconventional and microwave assisted.Here the establishment of physic chemical describer is a good tool for the prediction of antimicrobial activities.



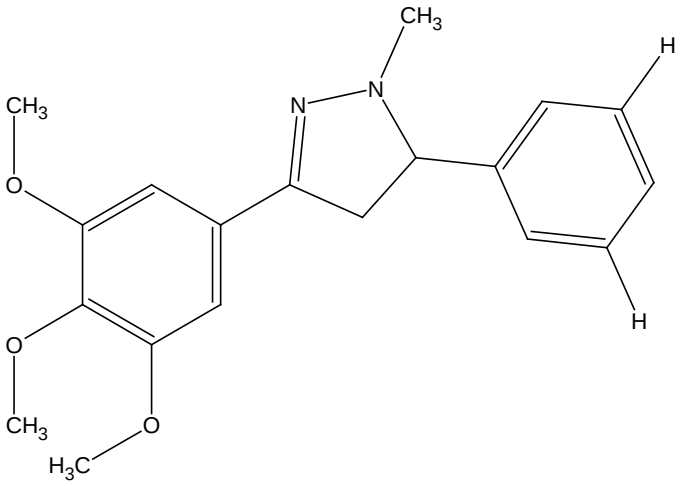
24. Rajarkur R. B et.al.,(2012) reported that study various chalcones were synthesized by the base catalyzed reaction between substituted aromatic ketones and substituted aromatic aldehydes. These chalcones were then subjected to the reaction with hydroxyl amine hydrochloride, guanidine hydrochloride and isoniazid to give 3,5- disubstituted isoxazoles, 4,6-disubstituted pyrimidine-2-amines and 3,5-disubstituted pyrazole derivatives respectively. These compounds were evaluated for their good antimicrobial, antifungal and antitubercular activity.



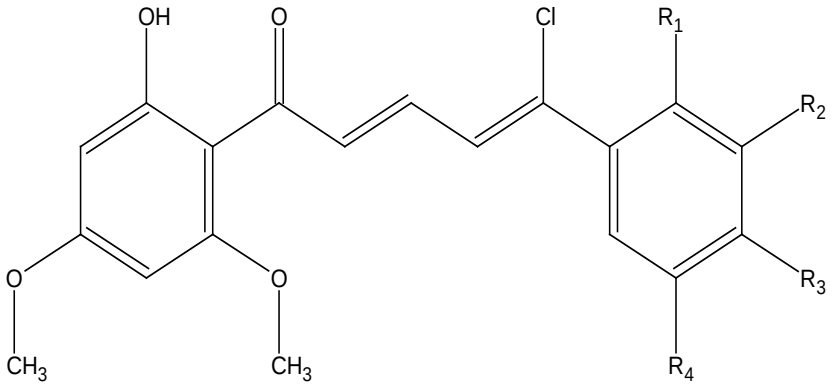
25. Naik A V et.al .,(2005) reported that, The chalcones are associated with different biological activities like insecticidal, anticancer, anti-inflammatory, bactericidal, fungicidal, antiviral, antitumor, antimalarial and antiulcer. Literature shows that lieochalcone and oxygenated chalcone has strong antileishmanial activity. It is reported that chalcones exhibited potent activity against human malarial parasite. Many workers have reported the different pharmaceutical activities of chalcones and its derivatives.



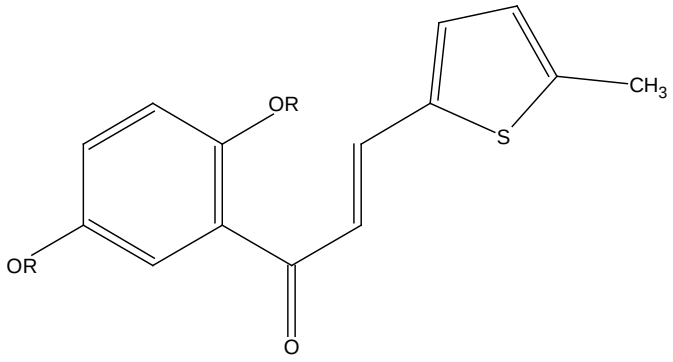
26. Rane et.al.,(2010) have reported synthesis of a acetylenic chalcones. The new acetylenic chalcones were evaluated for antimalarial and antitubercular activity. The antimalarial data for this series suggests that growth inhibition of the W2 strain of Plasmodium falciparum can be impacted by the introduction of a methoxy group ortho to the acetylenic group. Most of the compounds were active against Mycobacterium tuberculosis H37Rv.



27. Babasaheb et.al.,(2010)11 have reported synthesis and biological evaluation of β-chloro vinyl chalcones. All synthesized compounds were evaluated for their anti-inflammatory activity and antimicrobial activity. Most of compounds showed very good antibacterial and antifungal activity.

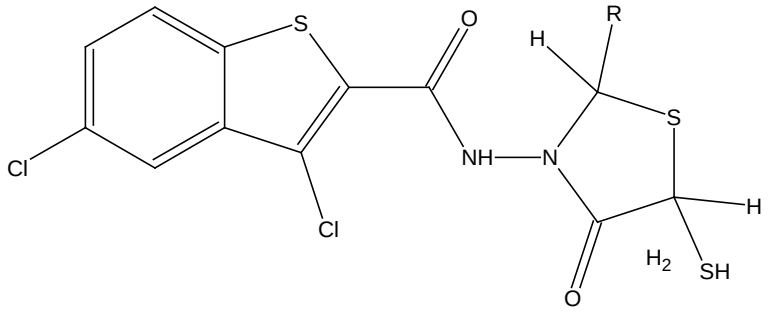


28. Jen-Hao et.al.,(2008)14 have reported synthesis of 2,5-dialkoxylchalcones. The new chalcones were prepared by Claisen–Schmidt condensation of appropriate acetophenones with suitable aromatic aldehyde. The novel 2,5-dialkoxylchalcones were evaluated for their cytotoxic, anti-inflammatory, anti-oxidant ,anti tubercular activity.

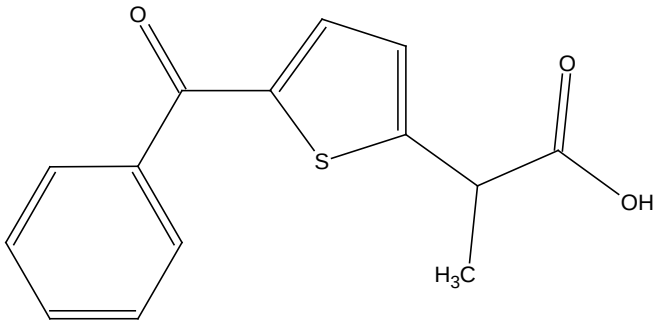


Literature review on thiophene nucleus

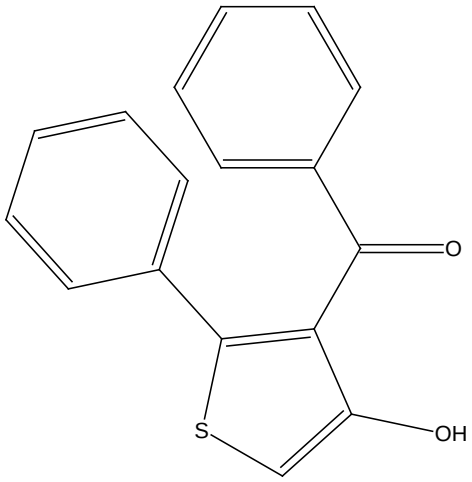
29.Thaker K M et.al.,(2007) reported that the synthesis of 4,thiazolidinone and 2,azetidinones bearing benzo(b) thiopjhene nucleus as potential anti tubercular and antimicrobial agents .In this anti tubercular evaluation of compound wsa carried out at tuberculosis antimicrobial acquisition co ordination facility(TAACF) .M.tuberculosis H37Rv to determined the actual minimum inhibitory concentration (MIC) in the BACTEC 460 the antitubercular activity data have been compared with standard drug rifampin at 0.25 microgram per ml. Concentration and it showed 98%inhibition .



30. Parvesh Singh P S et.al .,(1999) Sulphur compounds are of great chemical and pharmaceutical significance and display diverse properties such as antifungal, anti-HIV,antipsoratic,and antimicrobial activities. Some imidazo[2,1-b]-[1,3]thiazines and pyrimido[2,1-b]-[1,3]thiazines are well known anti-inflammatory agents. Likewise, thiophene compounds are well known to exhibit various biological and medicinal activities such as BACE1 inhibitors, antitubercular, anti-depressant, anti-inflammatory, anti-HIV PR inhibitors, and anti-breast cancer activities.

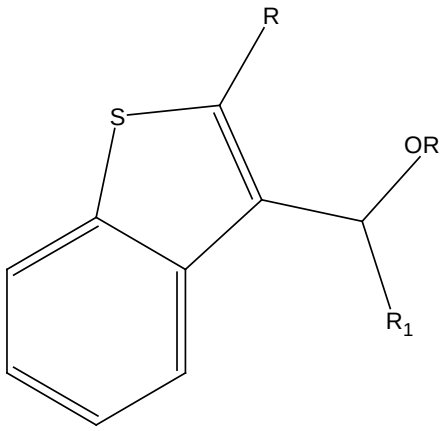


31. Ashish Das et.al., (2014) studied that A variety of biological and pharmacological importance of thiophene makes it an essential pharmacophore in the field of medicinal chemistry. Already marketed drugs like Clavix, Plavix which acts by irreversible inhibition of P2Y12 receptor, Tioconazole, a proven fungicidal agent which acts by inhibition of cell wall synthesis having thiophene core has been proven to be efficacious drugs in present respective disease scenario. Thus the synthesis and characterization of novel thiophene moieties with wider therapeutic consequences is a topic of interest for the medicinal chemist. This mini review enumerates the reported synthetic strategy to synthesize thiophene and its major therapeutic field as exploited in the literature.

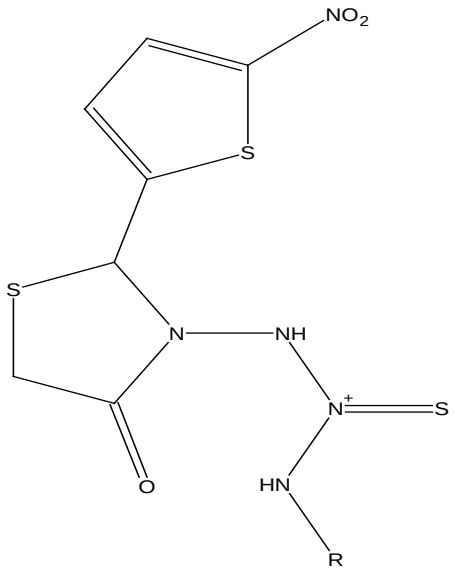


32. Raghav Mishr K K et.al.,(2011) studied that, Thiophene nucleus has been established as the potential entity in the largely growing chemical world of heterocyclic compounds possessing promising pharmacological characteristics. thiophenes significantly important

class of heterocyclic compounds and their applications in ever challenging chemotherapy of various ailments/ infections etc.



33. Sahar M I et.al .,(2010) It has been reported in the literature that heterocyclic compounds such as thiophenes exhibited potent anti-inflammatory activity. Likewise, thiazole, 1, 3, 4-thiadiazole, and their derivatives were found to possess anti tubercular and anti inflammatory activities.



3. AIM AND OBJECTIVE

AIM

The aim of this project is to discover molecules with potential anti -tubercular activity.

OBJECTIVE

Design compounds and docked against a specific crucial target, Methoxy Mycolic acid Synthase 2, which is involved in the cell wall synthesis. The synthesized compounds are expected to act on this enzyme.

THE PLAN OF WORK

- Design of methoxy mycolic acid synthase 2 inhibitors by docking studies.
- Insilico Drug likeness prediction.
- Insilico Toxicity Assessment.
- Laboratory synthesis of the compounds with top Docking Scores.
- Characterization of the synthesized compounds by
- Infrared Spectroscopy.
- H1 Nuclear Magnetic Resonance Spectroscopy.
- C13 Nuclear Magnetic Resonance Spectroscopy.
- Mass Spectroscopy.
- In-vitro anti -tubercular activity of synthesized compounds (MABA).

The whole study was carried out according to this flow chart

Identification of basic nucleus

↓

Structural Modification of the basic Nucleus

↓

Docking the molecule in the target protein

↓

Optimization of the highly Interacting molecule

↓

Top G score compounds selected

↓

Synthesis

↓

Characterization-Spectroscopy(IR,NMR,MASS)

↓

Acute toxicity studies

↓

Invitroanti tubercular activity

4. MATERIALS AND METHODS

DOCKING STUDIES

Drug design

Drug design is carried out using an automated docking program like GLIDE (grid based ligand docking with energetics) maestro 9.0 Schrodinger suites, Auto Dock or Argus Lab. It helps search molecules (ligands) having maximum favorable interactions with a receptor (target) usually a protein. Ligand is a single molecule whereas receptor may include proteins, metals and cofactors. It runs on rigid and flexible docking modes. The later which one generates conformations automatically for the input of each ligand and gives out the best fit pose of the molecule been docked on the receptor. [38]

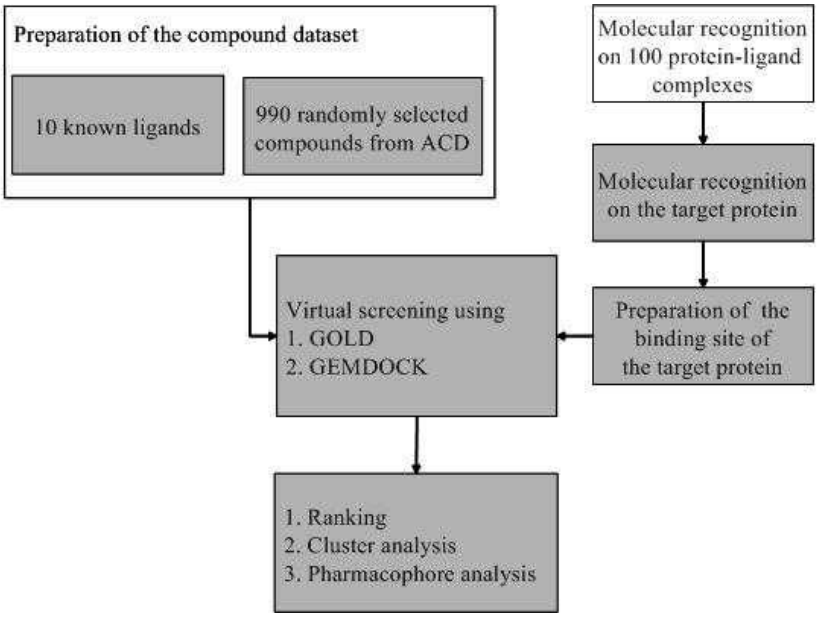
Types

There are two major types of drug design. The first is referred to as ligand based drug design and the second, structure based drug design.

1. Ligand based

Ligand based drug design (or indirect drug design) relies on knowledge of other molecules that bind to the biological target of interest. These other molecules may be used to derive a pharmacophore model that defines the minimum necessary structural characteristics. A molecule must possess in order to bind to the target. [39] In other words, a model of the biological target may be built based on the knowledge of what binds to it, and this model in turn may be used to design new molecular entities that interact with the target. Alternatively a quantitative structure activity relationship (QSAR), in which a correlation between calculated properties of molecules and their experimentally determined biological activity, may be derived. These QSAR relationships in turn may be used to predict the activity of new analogs.

Figure. 9



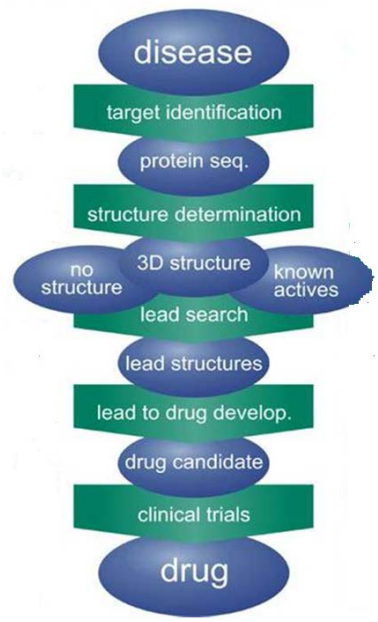
2. Structure based

Structure based drug design (or direct drug design) relies on knowledge of the three dimensional structure of the biological target obtained through methods such as x-ray Crystallography or NMR spectroscopy. [(40)] If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein. Using the structure of the biological target, Candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist. Alternatively various automated computational procedures may be used to suggest new drug candidates.

The 3D structures of bio molecular targets are obtained from X-ray Crystallography and NMR. In parallel, information about the structural dynamics and electronic properties about

ligands are obtained from calculations. This has encouraged the rapid development of the structure based drug design. (s

Figure. 10 (42)



COMPUTER-AIDED DRUG DESIGN

Computer-aided drug design uses computational chemistry to discover, enhance, or study drugs and related biologically active molecules. Molecular mechanics or molecular dynamics are most often used to predict the conformation of the small molecule and to model conformational changes in the biological target that may occur when the small molecule binds to it. Molecular mechanics methods may also be used to provide semi-quantitative prediction of the binding affinity. Also, knowledge-based scoring function may be used to provide binding affinity estimates.

Drug design with the help of computers may be used at any of the following stages of drug discovery,

1**. Hit identification** using virtual screening (structure- or ligand-based design)

2**. Hit-to-lead optimization** of affinity and selectivity (structure-based design, QSAR, etc.)

3**. Lead optimization**: optimization of other pharmacokinetic properties while maintaining affinity.

In order to overcome the insufficient prediction of binding affinity calculated by recent scoring functions, the protein-ligand interaction and a compound's 3D structure information are used for analysis. [43]

Active site identification

Active site identification is the first step in this program. It analyzes the protein to find the binding pocket, derives key interaction sites within the binding pocket, and then prepares the necessary data for Ligand fragment link. The basic inputs for this step are the 3D structure of the protein and a pre docked ligand in PDB format, as well as their atomic properties. Both ligand and protein atoms need to be classified and their atomic properties should be defined, basically, into four atomic types:

1. **Hydrophobic atom:** All carbons in hydrocarbon chains or in aromatic groups.

2. **H bond donor:** Oxygen and nitrogen atoms bonded to hydrogen atom(s).

3. **H bond acceptor:** Oxygen and sp2 or sp hybridized nitrogen atoms with lone electron pair(s).

4. **Polar atom:** Oxygen and nitrogen atoms that are neither H bond donor nor H bond acceptor, sulfur, phosphorus, halogen, metal, and carbon atoms bonded to heteroatom(s).[44]

DOCKING

Docking involves the fitting of a molecule into the target structure in a variety of positions, conformations and orientations. Molecular docking is used to predict the structure of intermolecular complex formed between two molecules. The small molecule called ligand

usually interacts with protein's binding sites.[45] Binding sites are areas of protein known to be active in forming of compounds. There are several possible mutual conformations in which binding may occur. These are commonly called binding modes. It also predicts the strength of the binding, the energy of the complex; the types of signal produced and calculate the binding affinity between two molecules using scoring functions.[46]

TYPES OF DOCKING

Lock and key or rigid docking- In lock and key docking, both the internal geometry of the receptor and ligand is kept fixed and docking was performed.

Induced fit or flexible docking- An enumeration on the rotations of one of the molecules (usually smaller one) is performed. For every rotation the surface cell occupancy and energy is calculated; later the most optimum pose was selected. [45]

ARGUS LAB

Argus lab 4.0 distributed freely and is made available for windows platforms by Planaria Software. It is an introductory molecular modelling package for academics. The Argus docking engine approximates an exhaustive search method with similarities to AUTO DOCK and GLIDE.

Flexible ligand docking possible with Argus lab where the ligand is described as torsion tree or free and grids are constructed that overlay the binding site. The key features such as "the nature of binding site and the number of rotatable bonds to the ligand, can be determined. [46]

STEPS INVOLVED IN DOCKING

Docking is done by using ARGUS LAB Software.

1. Protein preparation.

2. Selection of active site (Q-Site finder).

3. Ligand Preparation.

4. Docking Procedure.

5. Visualization / Interpretation of Docking.

1. PROTEIN PREPARATION

Step: 1

- Enter protein (pdb) ID in the protein data bank. (1TPY)
- Go to download files and select pdb as text file.
- Save the download pdb (text file) to the desktop.

Step: 2

- Open Argus lab file Open Import pdb file from the desktop.
- 3D Structure of the protein will appear in the workspace of Argus lab.
- Left side of the screen shows molecular tree view.
- Open pdb Open 'residues' 'Open misc'
- From 'Misc' delete the inhibitor and hetero residues [Note: Do not delete Co factor]
- Open water press shift, select all water molecules and delete.
- Add hydrogen atoms.
- Go to Calculation on the toolbar energy by UFF method start.
- Save the prepared protein as *.agi file format in the desktop.

2. Q-SITE FINDER [47]

Step: 1

- Open Q-Site finder through online.
- Upload / Import the PDB format of the Protein.

- Find all the active site and make a list out of the common amino acid residues.

Step: 2

- Open residues open Amino acids.
- Press control and select the amino acids which were listed from the Q-Site finder.
- Make sure that all amino acid residues listed are selected.
- Right click on the mouse make a group from the selected residues give name Binding site Ok.

3. LIGAND PREPARATION

- Draw the structure from Chem sketch and save as MDL Mol format.
- Import the ligand into workspace of Argus lab.
- Clean Geometry Clean Hybridization.
- Select the ligand, Right click on the mouse Make a group from the residues give name ligand Ok.

4. DOCKING PROCEDURE

- Select the set up a Dock Ligand calculation from the toolbar.
- Argus Dock as the Docking Engine.
- Dock was selected as calculation type.
- Flexible for the scoring function.
- Calculation size.
- Start docking.
- Save the Docked protein Ligand complex as Brookhaven pdb files (*.pdb).

5. VISUALIZATION / INTERPRETATION OF DOCKING

- Molegro Molecular viewer will help in analyzing the energies and interaction of the binding.
- View Docking view & Secondary Structure view.
- View Hydrogen bond interaction.
- Ligand map Interaction overlay.

IN-SILICO SCREENING OF DRUG LIKENESS

A drug to be pharmacologically active and exert the action it should possess Pharmacokinetic properties like absorption, distribution, metabolism and excretion. In the field of drug research and development many drug failures occur due to unfavorable ADME properties. This has to be ruled out earlier in the process of drug discovery. Some computational methods have been evolved to investigate the most suitable drug molecules before synthesis. [48]

"Lipinski's rule of five" it is also known as Pfizer's rule of five is rule to evaluate drug likeness. It is used to predict whether a molecule is likely to be orally bio-available or to evaluate drug likeness.

Lipinski's rule

Lipinski's rule is used to predict if a molecule is likely to be orally bio-available or to evaluate drug likeness. The rule was formulated by Christopher A. Lipinski in 1997. The rule states that for drug likeness the molecule should have the following properties.

- Molecular weight less than 500 Daltons.
- Calculated log P value less than 5.
- Less than 10 hydrogen bond acceptor groups(e.g.-O-,-N-,etc).
- Less than 5 hydrogen bond donor groups (eg .NH ,OH etc).

- Less than 10 rotatable bonds.

The designed and docked molecules are screened insilico using Molinspiration Cheminformatics software to evaluate drug likeness. This tool is quick and easy to use. It is a software available online for calculation of important molecular properties (log P, polar surface area, number of hydrogen bond donors and acceptors and others),as well as prediction of bioactivity score for the most important drug targets(GPCR ligands, kinase inhibitors, ion channel modulators, nuclear acceptors. [49]

ADME ANALYSIS

A deeper understanding of the relationships between important ADME parameters and molecular structure and properties has been used to develop in silico models that allow the early estimation of several ADME properties. Among other important issues, prediction of properties that provide information about dose size and dose frequency such as oral absortion, bioavailability, brain penetration clearance and volume of distribution (for frequency) also needed. [50]

Absorption

A compound crossing a membrane by purely passive diffusion, a reasonable permeability estimate can be made using single molecular properties, such as log D (diffusion co-efficient) or hydrogen- bonding capacity. The simplest Insilico models for estimating absorption are based on a single descriptor, Such as log P (partition coefficient) or log D, or polar surface area, which is a descriptor of hydrogen – bonding potential. Different multivariate approaches such as multiple liner regressions, partial least squares and artificial neural networks, have been used to develop quantitative structure-human-intestinal-absorption relationships.

Bioavailability

Important properties for determining permeability seem to be the size of the molecule, as well as its capacity to make hydrogen bonds, its overall lipophilicity and possibly its shape and flexibility.

Blood –Brain Barrier penetration (BBB)

Drugs that act in the CNS need to cross the blood-brain barrier (BBB) to reach their molecular target by contrast, for drugs with a peripheral target, little or no BBB penetration might be required in order to avoid CNS side affections. Rule –of –five like recommendations regarding the molecular parameters that contribute to the ability of molecules to cross the BBB have been made to aid BBB- penetration predictions; for example molecules with a molecular mass of <450Da or with polar surface area (PSA) <100A0 are more likely to penetrate the BBB.

Dermal and ocular penetration

The existing transdermal models are typically a function of the octanol/water partition coefficient and dermas that have been associated with aqueous solubility, including hydrogen –bonding parameters, molecular weight and molecular flexibility. Commercial models for the prediction of solute –permeation rates through the skin are available, for example qikrop and Derm Win programs.

METABOLISM

Insilico approaches to predicting metabolism can be divided into QSAR and three dimensional –QSAR studies, protein and pharmacophore models and predictive database. Some of the first –generation predictive –metabolism tools currently require considerable input from a computational chemist, whereas others can be used as rapid filters for the screening of virtual libraries. Perhaps the most intellectually satisfying molecular modeling studies are those based

on the crystal structure of the metabolizing enzymes. Ultimately, such programs might be linked to computer-aided toxicity prediction on the basis of quantitative structure-toxicity relationships and expert systems for toxicity evaluation. (51)

TOXICITY PREDICTION

All the data set molecules were subjected to the toxicity risk assessment by using Osiris program, which is available online. The OSIRIS property Explorer shown in this page is an **integral** part of Actelion's in house substance registration system. It allows drawing chemical structures and also calculates various drug relevant properties whenever a structure is valid. Prediction results are color coded in which the red color shows high risks with undesired effects like mutagenicity or a poor intestinal absorption and green color indicates drug-conform behavior. (52)

Molecular property prediction includes

- Toxicity risk assessment
- clog P prediction
- Solubility prediction
- Molecular weight
- Drug likeness prediction

SYNTHETIC METHODOLOGY

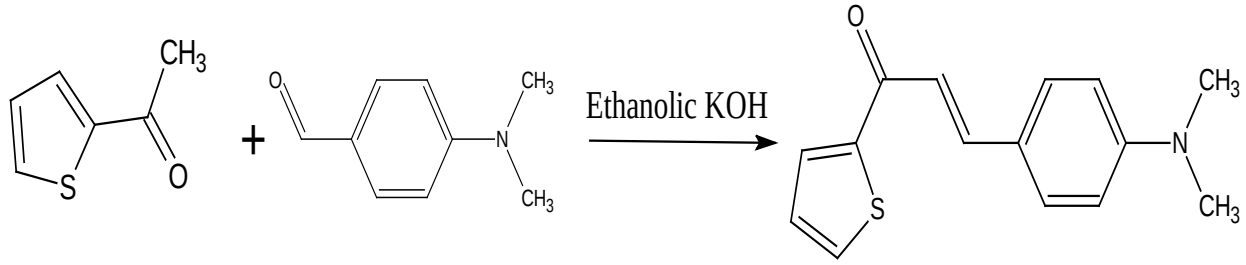
SYNTHESIS

Synthetic scheme was framed for the hit compounds from docking and the procedure for synthesis was collected from literatures. The necessary chemicals of laboratory grade for the synthesis where procured from Sigma Aldrich and Synthesis was carried out.

Scheme 1[53]

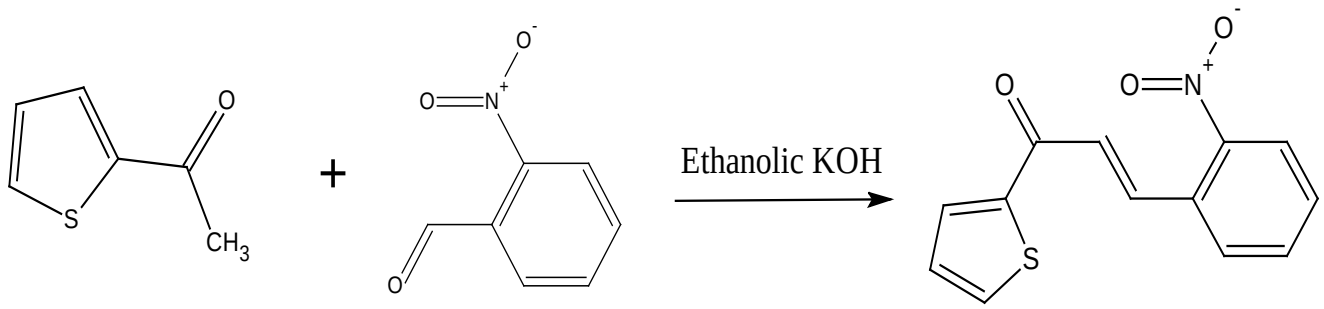
2-Acetyl thiophene (0.01mol) and appropriately substituted N, N Dimethyl amino benzaldehyde (0.012 mol) were mixed in ethanol (20ml) containing 10% aq. Potassium hydroxide (8ml) and magnetically stirred the solution constantly at room temperature for 10 hours. The whole mixture was transferred in to 100ml ice cold water and acidified with dil.Hydrochloric acid.The solid form was washed, filtered and dried, recrestallised from absolute ethanol.

Compound 1



Compound 2:

2-Acetyl thiophene (0.01mol) and appropriately substituted O-Nitro benzaldehyde (0.012 mol) were mixed in ethanol (20ml)containing 10% aq.potassium hydroxide (8ml) and magnetically stirred the solution constantly at room temperature for 10 hours . The whole mixture was transferred in to 100ml ice cold water and acified with dil.Hydrochloric acid.The solid form was washed, filtered and dried, recrestallised from absolute ethanol.

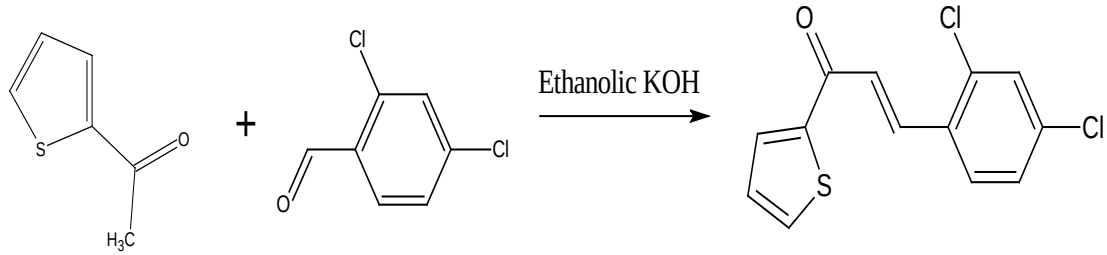


Compound 3:

The compound was synthesized by two a step reaction.

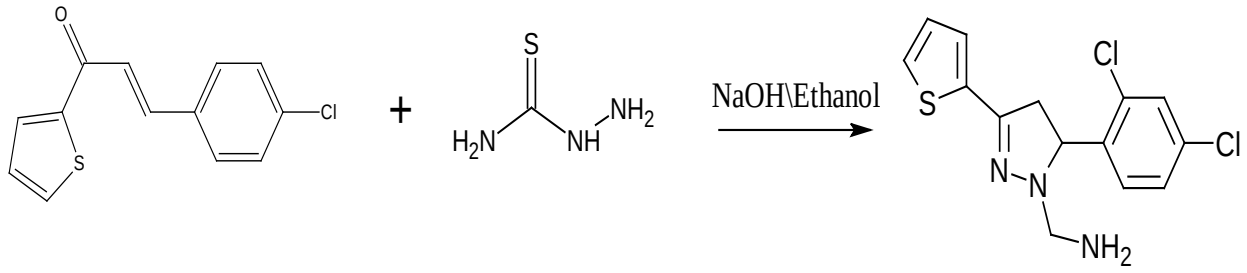
Step1: [53]

2-Acetyl thiophene (0.01mol) and appropriately substituted 2,4 Di Chloro benzaldehyde (0.012 mol) were mixed in ethanol (20ml)containing 10% aq. Potassium hydroxide (8ml) and magnetically stirred the solution constantly at room temperature for 10 hours . The whole mixture was transferred in to 100ml ice cold water and acified with dil.Hydrochloric acid.The solid form was washed, filtered and dried , recrestallised from absolute ethanol .



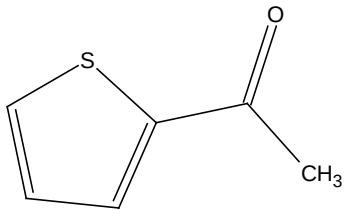
Step 2[(54)]

A mixture of chalcone (0.02mol),thiosemicarbazide (0.02mol)were dissolved in ethanolic sodium hydroxide solution (10ml) was stirred for 3hrs.then it was poured into 400ml of cold water with continuous stirring for 1hour then left overnight. The precipitate formed was filtered, washed and recrystallised from ethanol.



REACTANT PROFILE

2 Acetyl thiophene



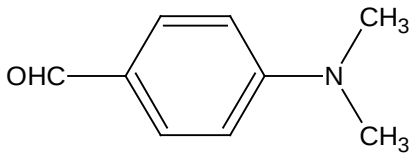
Molecular formula : C6H6OS

Molecular weight : 126.18

Appearance : Light yellowish liquid

Melting point : 10-11

N, N Dimethyl amino benzaldehyde



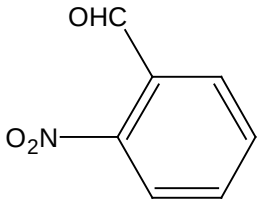
Molecular formula : C9H11N0

Molecular weight : 149.19

Appearance : Yellow white powder

Melting point : 74°c

O-Nitro benzaldehyde



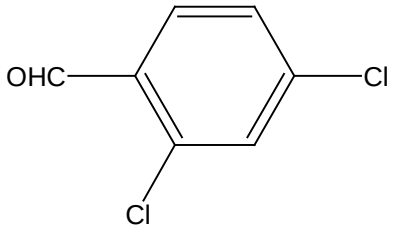
Molecular formula : C7H5NO3

Molecular weight : 151.11

Appearance : Pale yellow crystalline powder

Melting point : 41-44°c

2, 4 Di chloro benzaldehyde



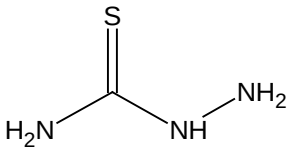
Molecular formula : C7H4Cl2O

Molecular weight : 175.01

Appearance : White crystalline solid

Melting point : 64-69°C

Thiosemicabazide



Molecular formula : CH5N3S

Molecular weight : 91.14

Appearance : White crystalline solid

Melting point : 177-179°C

RECRYSTALLISATION:

Ethanol was added to the synthesised compounds and heated until it dissolved completely .The clear solution thus obtained was filtered immediately and set aside for cooling .On cooling crystals gradually appeared.

CHARACTERISATION STUDIES

MELTING POINT

The melting point of the synthesized compound was determined by tone end open capillary tube method. The temperature at which the compound starts losing its crystallinity and changes from solid to liquid form was found recorded.

IR SPECTROSCOPY

IR spectroscopy helps to ascertain the presence and absence of the functional group. The synthesized compound was made into a pellet with potassium bromide by pressed pellet technique using pellet press (Model No: M15). The pellet was mounted on the pellet disc and percentage transmittance was recorded in ABB IR Spectrophotometer (Model No: 3000). IR Spectroscopy is an important tool for structure elucidation and compound identification.

EXAMPLE:

- OH groups : 3600-3200 cm^{-1}
- C=Ogroups : 1710 cm^{-1}
- Ar C-H str : 3050 -3000cm^{-1}
- C=C str : 1600 cm^{-1}
- N-S str : 3540-3300 cm^{-1}
- C-H aliphatic str : 2795-2840 cm^{-1}

NMR SPECTROSCOPY

Proton NMR Spectroscopy helps us to study the number of equivalent protons and their environment thereby we can ascertain the structure of the molecule. The NMR spectra was recorded on 300 MHz BRUKER Advance III NMR Spectrometer DMSO was used as a solvent.

MASS SPECTROSCOPY

Mass Spectra was recorded on Shimadzu HPLC-MS using Electron Spray Ionization

Technique and was quantified using Lab Solutions Software 7.0, Samples were prepared by dissolving a minute quantity of pure compounds in methanol. The fragmentation patterns were reported in m/z values. [55]

HYPHENATED TECHNIQUE

GC-MS: To determine the mass and also get an idea about the purity of the sample.

BIOLOGICAL EVALUATION

ANTI-TB ACTIVITY

There are various high through put assays available for screening of new chemical entities against Tuberculosis. They are

- Microplate Alamar blue Assay
- BACTEC Assay
- Luciferous reporter phage assay
- REMA Assay
- Broth Dilution Assay
- Middle brook (7H9,7H10,7H11) Agar Dilution Assay

PRINCIPLE

The micro plate Alamar blue assay (MABA) is an indirect colorimetric DST method for determining the MICs of TB drugs for strains of mycobacterium tuberculosis . in this assay , the redox indicator alamar blue monitors the reducing environment of the living cell . It turns from blue to pink in the presence of mycobacterial growth.

PROCEDURE

- The anti-mycobacterial activity of compounds (M13,M14) were assessed against M.tuberculosis using micro plate Alamar blue assay(MABA)
- This methodology is non – toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.
- Briefly, 200ml of sterile de-ionised water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.

- The 96 wells plate received 100 ml of the middle brooke 7H9 broth and serial dilution of compounds was made directly on a plate.
- The final drug concentrations tested were 100 to 0.8mg/ml
- Plates are covered and sealed with paraflim and incubated at 37c was dive days
- After this time 25 ml of freshly prepared 1:1 mixture of alamar blue reagent and 10% tween 80was added to the plate and incubated for 24 hours.
- A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth.
- The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink .[56]

ADVANTAGES

- It has accurate time-course measurement
- It has highly sensitivity and linearity
- It is ideal for use with post-measurement functional assays
- It is flexible and it can be used with different cell modes
- It is scable and it can be used with flourescence and /or absorbance-based instrumentation platforms
- It is non toxic , on –radioactive and is safe for the user,

APPLICATIONS

- Especially meant for studies on Mycobacterium tuberculosis
- Used extensively in cell viability and cytotoxicity.

5. RESULTS AND DISCUSSION

Nearly 200 molecules were sketched using chemsketch. Were docked against the enzyme using A. L 4.0.1 Software. The molecules were also docked against the following targets. The molecule with best docking score and interaction were selected and synthesis.

The molecules were also docked against the following targets:

1. Methoxy mycolic acid synthase 2

2. Glutamine synthatese 1

3. Cyclopropane mycolic acid synthase 2

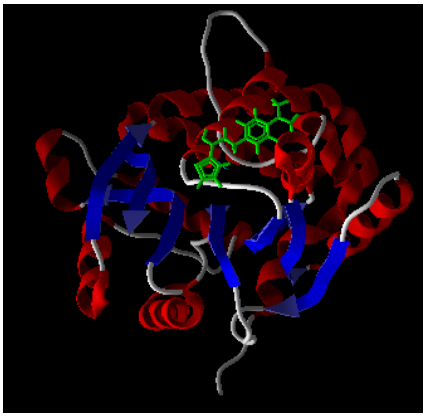
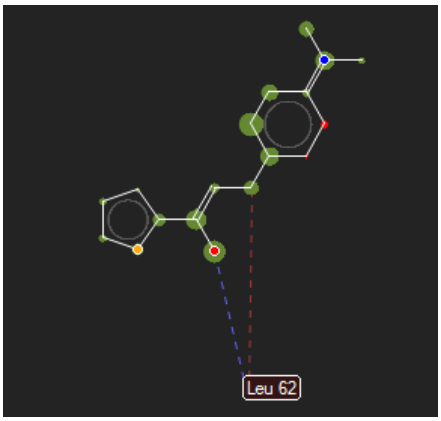
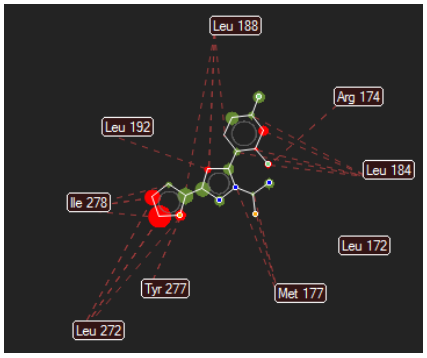
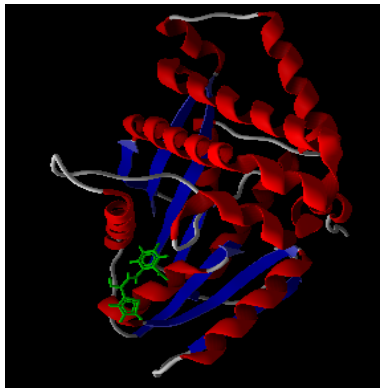
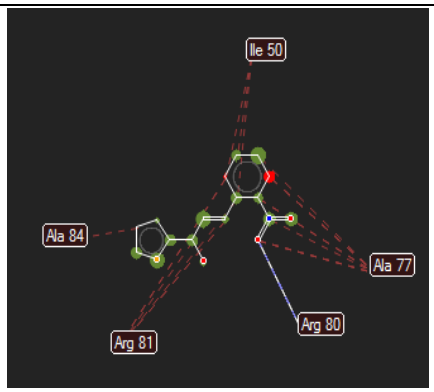
4. Decaprenylphosphoryl-b-d-ribose2'-Epimerase1 (DprE1)

Table no: 1

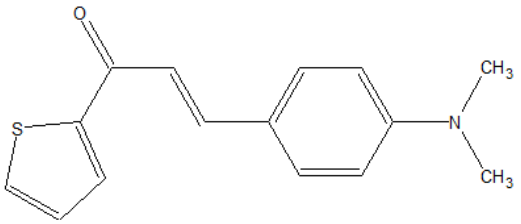
NAME OF THE ENZYME	DOCKING SCORE (K cal/ mol)		
	DKS1	DKS2-1	DKS3
Methoxy mycolic acid synthase 2	-10.67	-10.76	-8.30
Glutamine synthatase 1	-10.53	-9.45	-8.43
Cyclopropane Mycolic Acid synthase 2	-9.56	-10.72	-9.0
L.D Transpeptidase	-9.78	-10.72	-7.2

INTERACTIONS OF THE DOCKED MOLECULE USING THE ENYME METHOXY MYCOLIC ACID SYNTHASE 2

Table no: 2

SAMPLE CODE	DOCKING VIEW	INTERACTION WITH AMINO ACIDS
DKS1		
DKS2-1		
DKS3		

RESULTS OF SCHEME
COMPOUND NAME: DKS1



IUPAC NAME: (2*E*)-3-[4-(dimethylamino)phenyl]-1-thien-2-ylprop-2-en-1-one

Molecular formula : C15H15NOS

Molecular weight : 257.35g/mol

Appearance : Reddish orange

Melting point : 69°c

Composition : C(70.01%)H(5.87%)N(5.44%)O(6.22%)S(12.46%)

Molar refractivity : 79.80±0.3cm^3

Molar volume : 217.2±3.0cm^3

Surface tension : 49.4±3.0dyne/cm

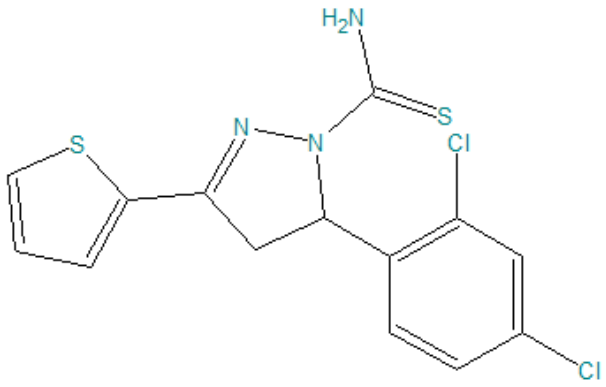
Density : 1.184±0.06g/cm^3

Parachor : 31.63±0.5,10-24cm^3

Index of refraction : 1.655±0.02

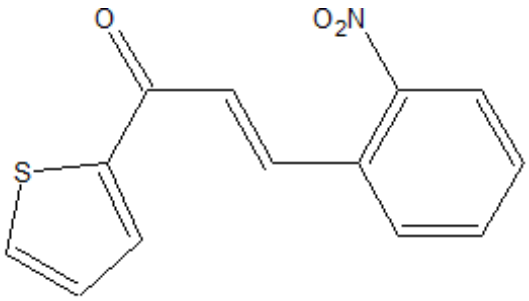
Polarizability : 31.63±0.5,10-24cm^3

COMPOUND NAME: DKS2-1



IUPAC NAME	:	5-(2,4-dichlorophenyl)-3-thien-2-yl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide
Molecular formula	:	C14H11CL2N3S2
Molecular weight	:	356.29
Appearance	:	yellowish brown
Melting point	:	72°c
Composition	:	C(47.19%)H(3.11%)C(19.90%)N(11.79%)S(18.00%)
Molar refractivity	:	92.68±0.5cm^3
Molar volume	:	226.5±7.0cm^3
Surface tension	:	58.8±7.0dyne/cm
Density	:	1.57±0.1g/cm^3
Parachor	:	627.5±8.0cm^3
Index of refraction	:	1.754±0.05
Polarizability	:	36.74±0.5,10-24cm^3

COMPOUND NAME: DKS3



IUPAC NAME	:	(2*E*)-3-(2-nitrophenyl)-1-thien-2-ylprop-2-en-1-one
Molecular formula	:	C13H9NO3S
Molecular weight	:	259.38
Appearance	:	Light brown
Melting point	:	67°c
Composition	:	C(60.22%)H(3.50%)N(5.40%)O(18.51%)S(12.37%)
Molar refractivity	:	72.03±0.3cm^3
Molar volume	:	191.1±3.0cm^3
Surface tension	:	58.9±3.0dyne/cm^3
Density	:	1.346±0.06g/cm^3
Parachor	:	529.6±4.0cm^3
Index of refraction	:	1.677±0.02
Polarizability	:	28.55±0.5,10-24cm^3

IR SPECTROSCOPY

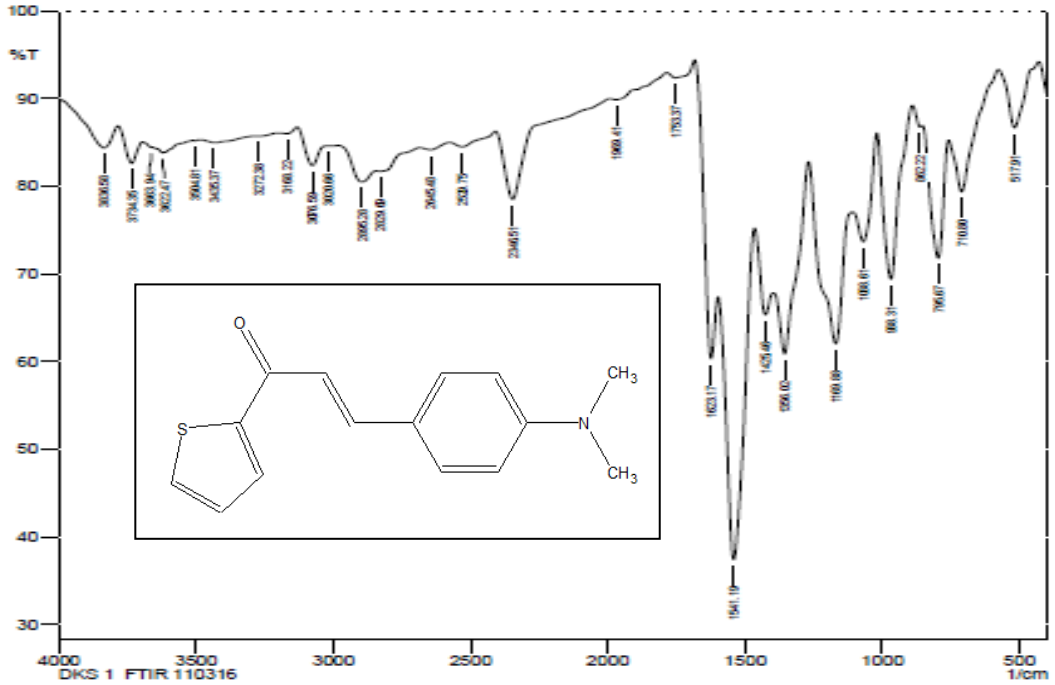
The sample were prepared by the KBr pellet techniques of spectrum. The spectra were examined for the absence of the functional groups of the parent compounds and examined for the presence of the vibrational absorption band for the new functional group.

The synthetic reaction involves, two compounds invoved in the reaction between ketone and aldehydes to yield chalcones . Another one compound invoved in the reaction between chalcones and thiosemicarbazide to yield benzimidazole derivative.

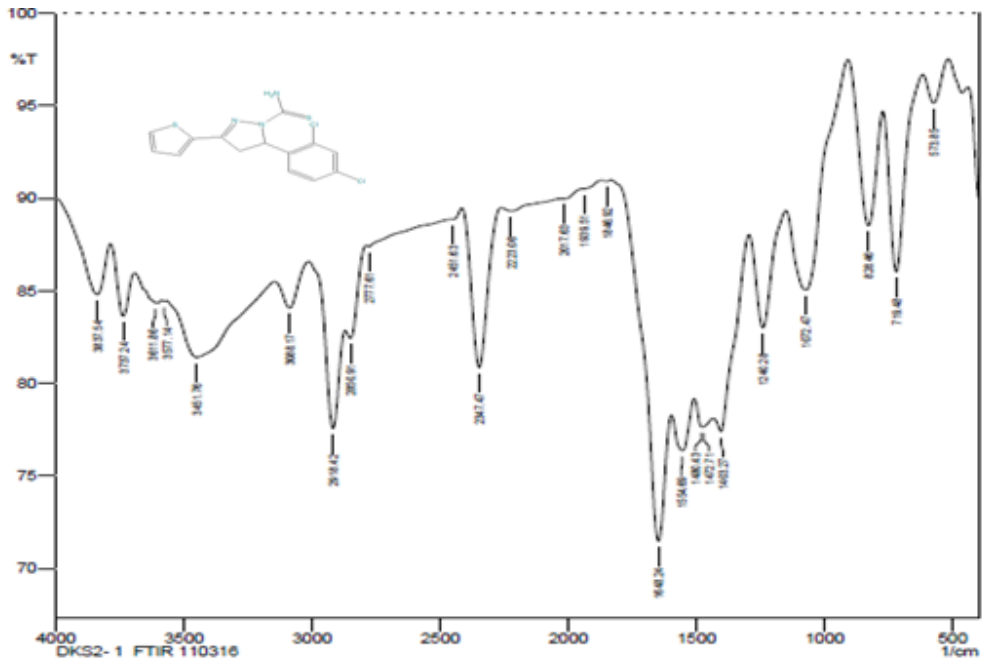
Table no :3

ABSORBANT BAND	**DKS1**	**DKS2-1**	**DKS3**
C=0 Stretching	✓	✓	✓
Ar C-H Stretching	×	✓	×
C=C Stretching	✓	✓	✓
N=N Stretching	×	✓	×

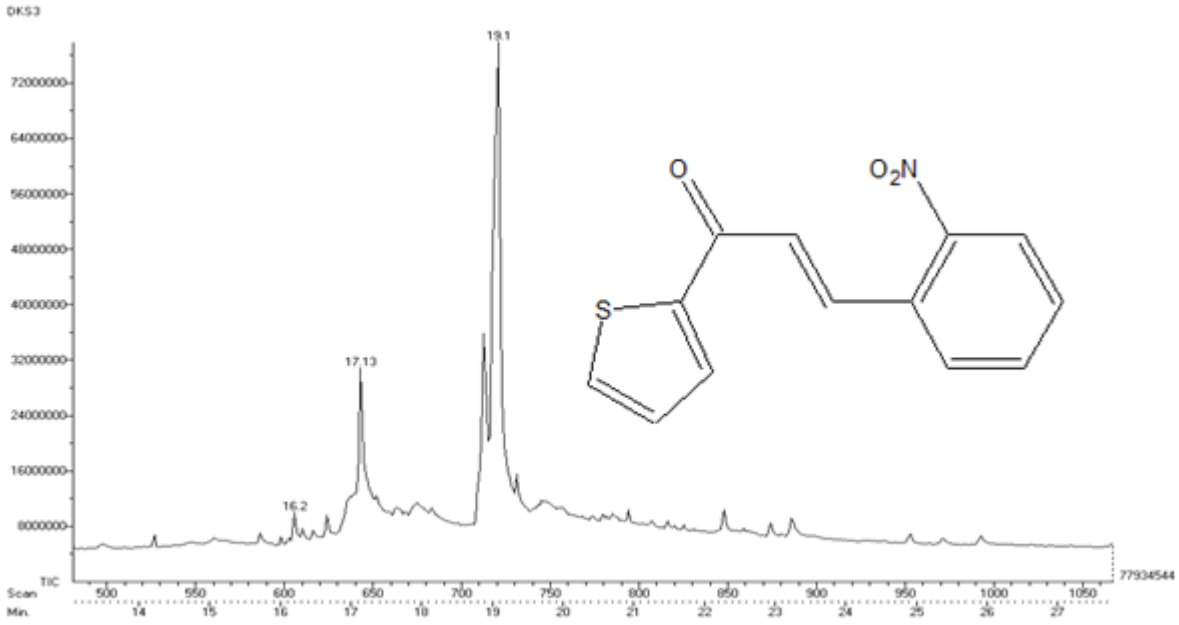
SAMPLE CODE : DKS1



SAMPLE CODE : DKS2-1



SAMPLE CODE : DKS3



NMR SPECTROSCOPY

SAMPLE CODE :DKS1

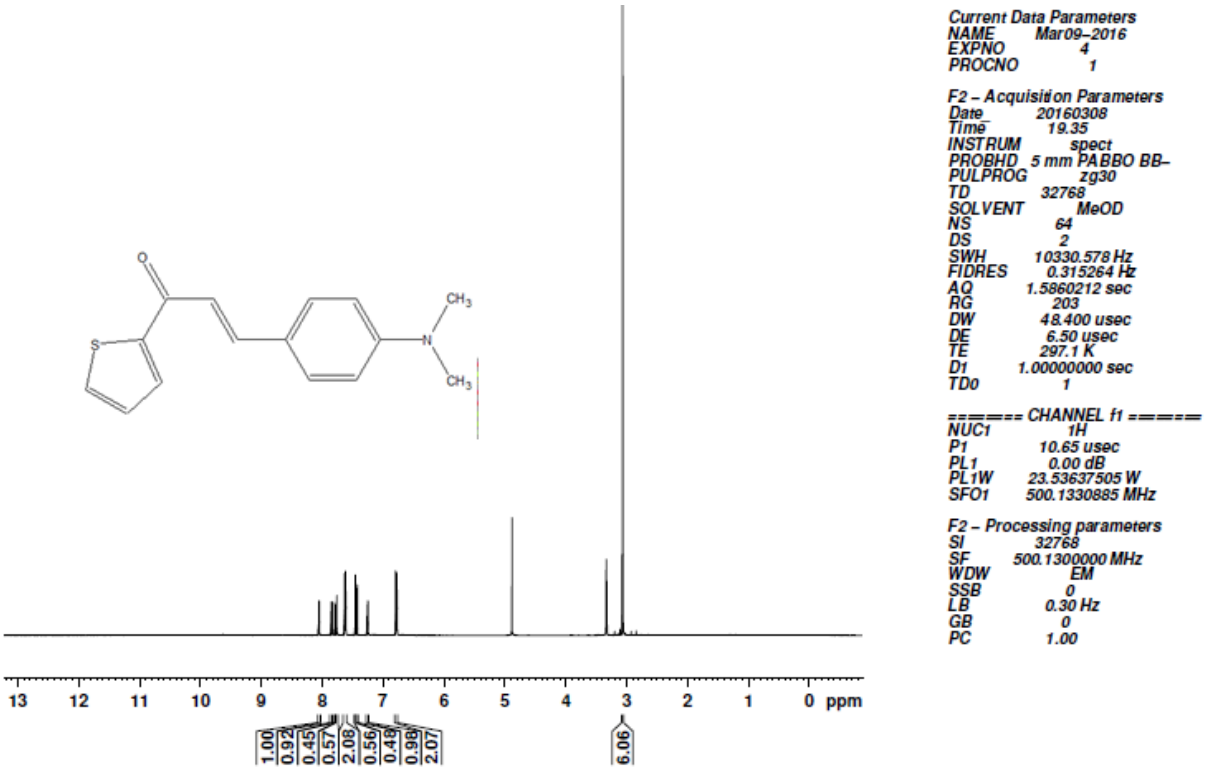


Table no. 4

S.NO	§ VALUE	NATURE OF PEAK	NUMBER OF PROTONS
1	6.62	Doublet	2 protons
2	6.70	Doublet	2 protons
3	7.24-7.25	Multiplet	3 protons
4	7.42	Singlet	1proton
5	7.45	Singlet	2protons
6	7.60	Doublet	2 protons
7	7.75	Singlet	2protons
8	8.04-8.05	Doublet	1proton

SAMPLE CODE : DKS2-1

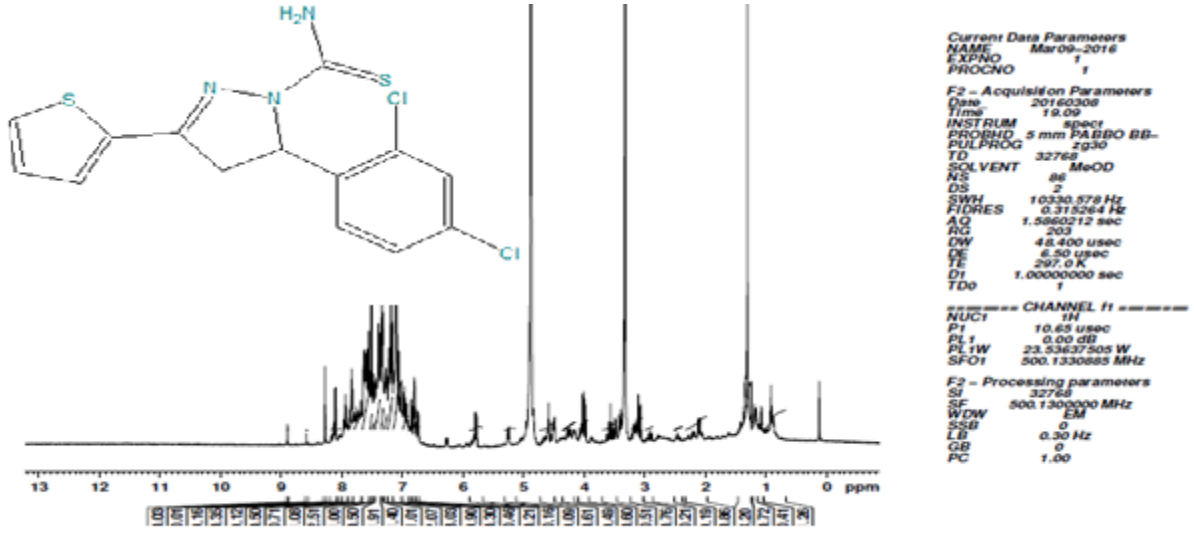


Table no: 5

S.NO	§ VALUE	NATURE OF PEAK	NUMBER OF PROTONS
1	7.84 ppm	Triplet	1proton
2	7.85ppm	Singlet	1proton
3	7.90-7.97ppm	Multiplet	1proton
4	7.33-7.23	Multiplet	3protons
5	7.17-7.22	Multiplet	3protons
6	1.166-1.22	Multiplet	1proton
7	o.871-0.937	Multiplet	1proton

SAMPLE CODE : DKS3

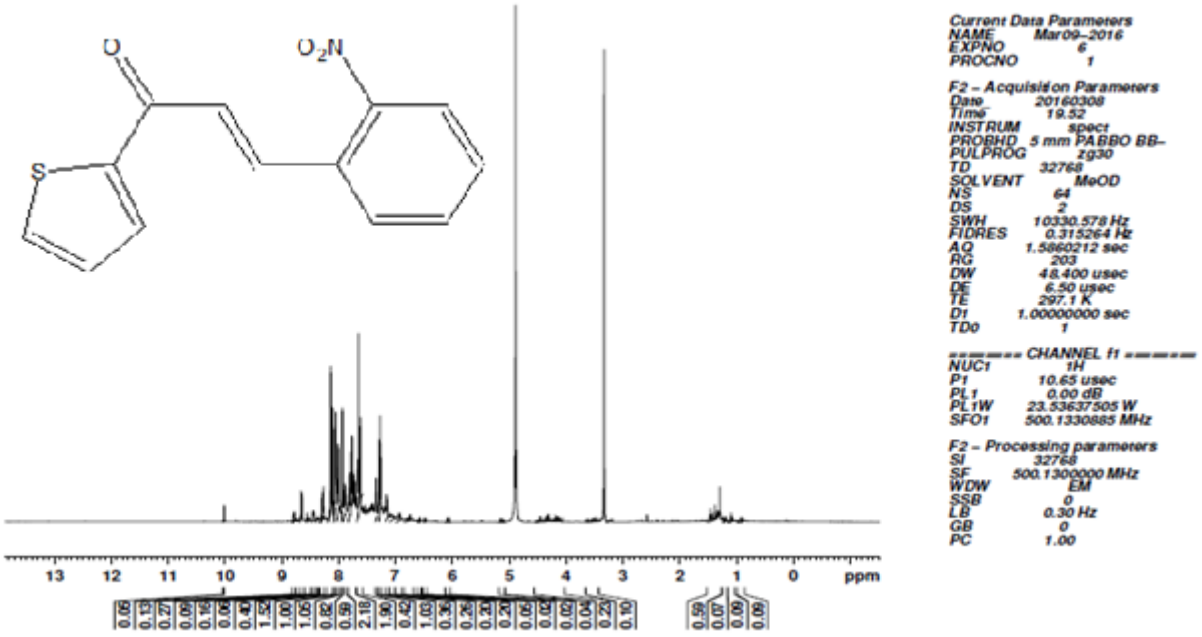


Table no: 6

S.NO	§ VALUE (PPM)	NATURE OF PEAK	NUMBER OF PROTONS
1	8.05ppm	Doublet	1 proton
2	7.55-7.78ppm	Multiplet	1proton
3	7.80-7.84ppm	Multiplet	2proton
4	7.87 ppm	Doublet	1proton
5	7.95ppm	Doublet	1proton
6	7.36ppm	Doublet	2proton
7	1.34ppm	Triplet	1proton

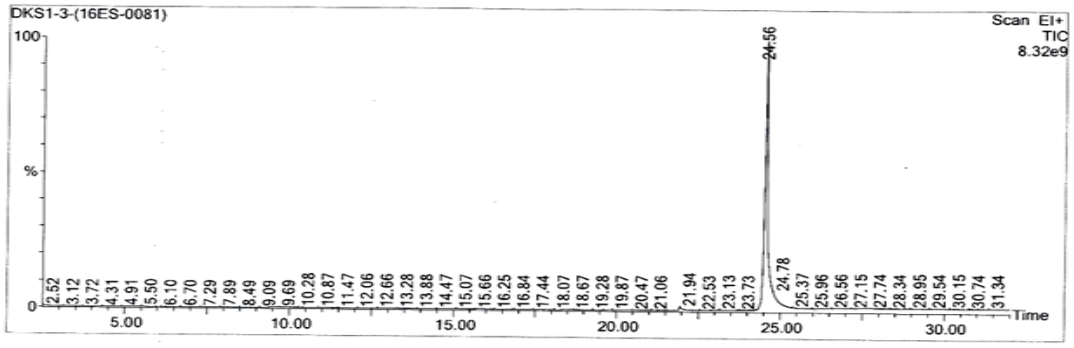
GC-MS SPECTROSCOPY

The molecular weight of the synthesised compounds were comfirmed by GC-MASS analysis

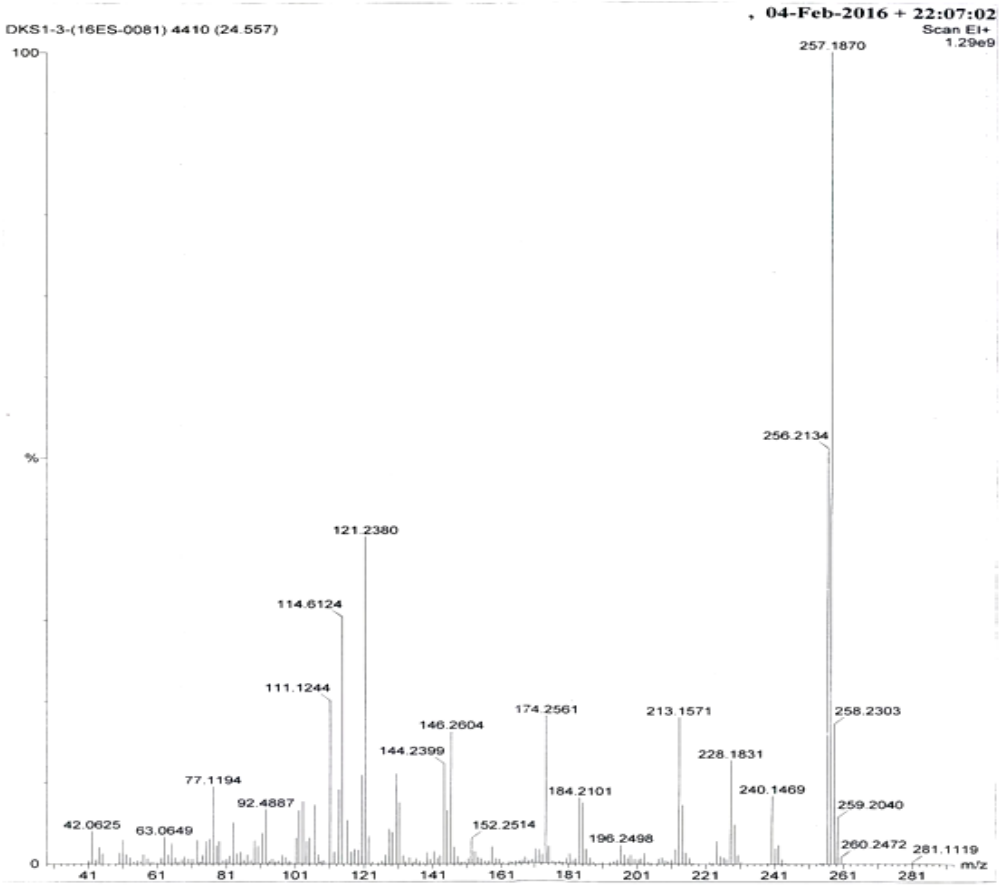
Table no:7

SAMPLE CODE	CALCULATED MASS	ACTUAL MASS
DKS1	257.35g/mol	257.0 g/mol
DKS2-1	352.29g/mol	352.20g/mol
DKS3	259.38g/mol	259.27g/mol

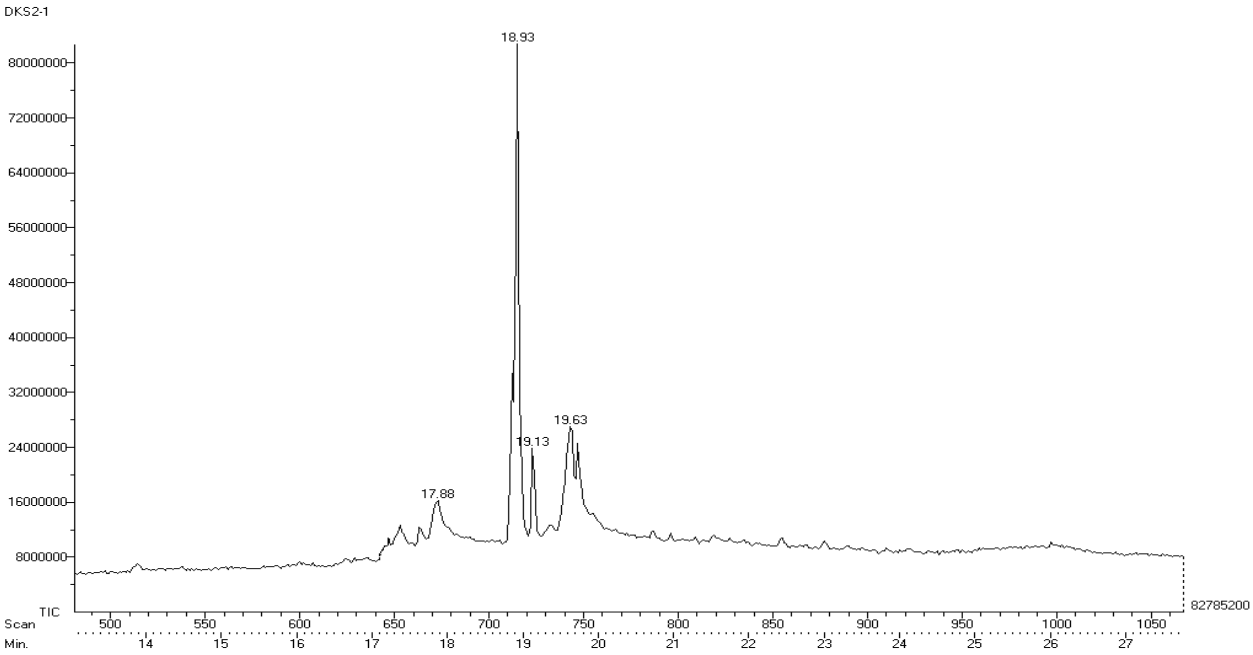
SAMPLE CODE : DKS1

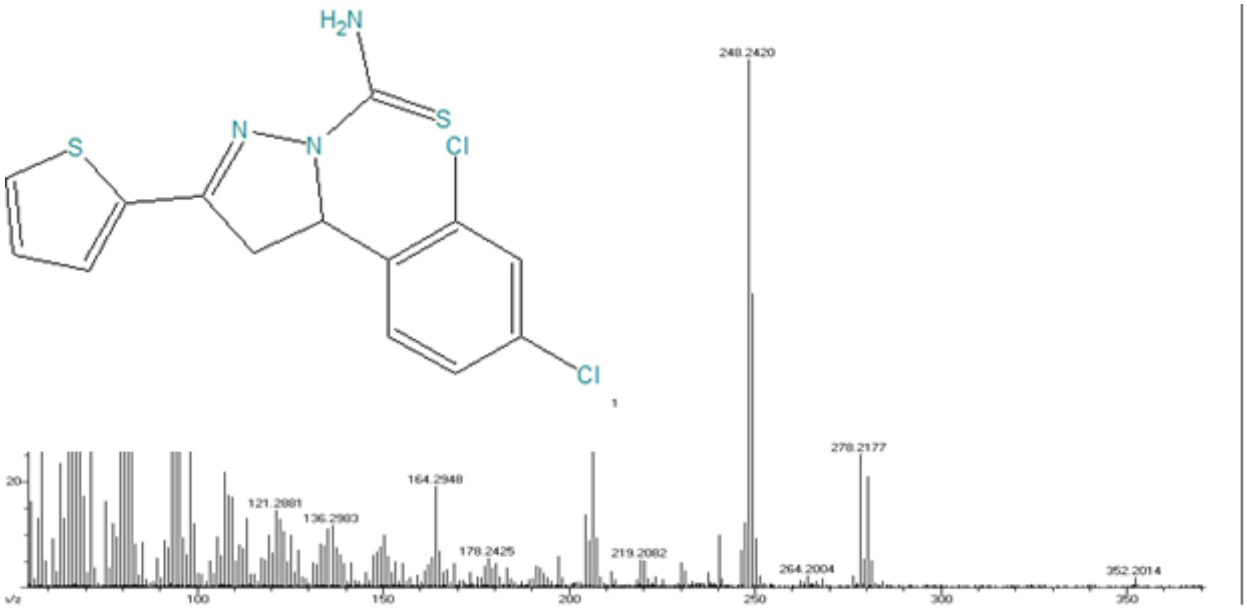


#	RT	Scan	Height	Area	Area %	Norm %
1	21.936	3886	99,814,072	10,130,416.0	1.171	1.19
2	24.557	4410	8,147,079,680	854,759,936.0	98.829	100.00

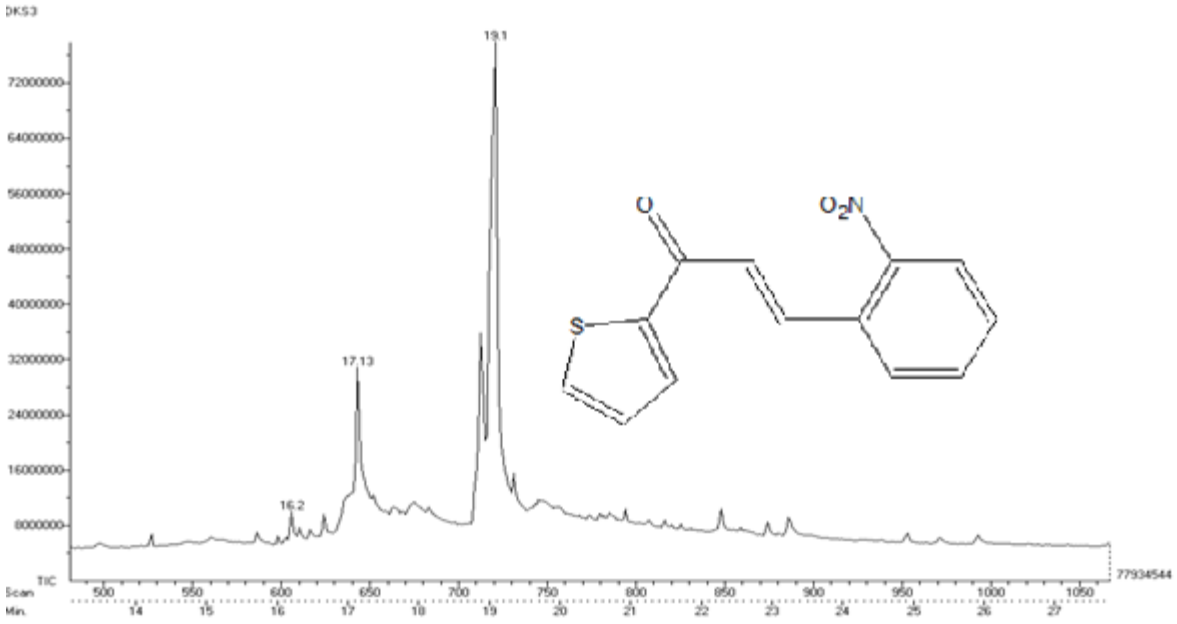


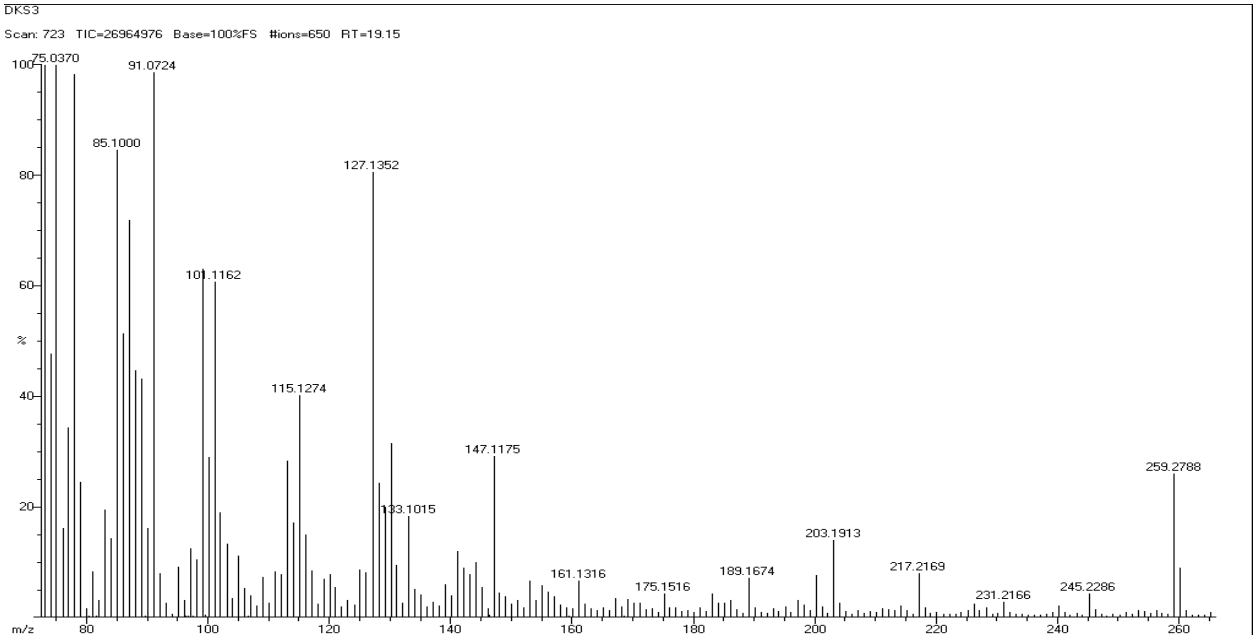
SAMPLE CODE :DKS2-1





SAMPLE CODE : DKS3





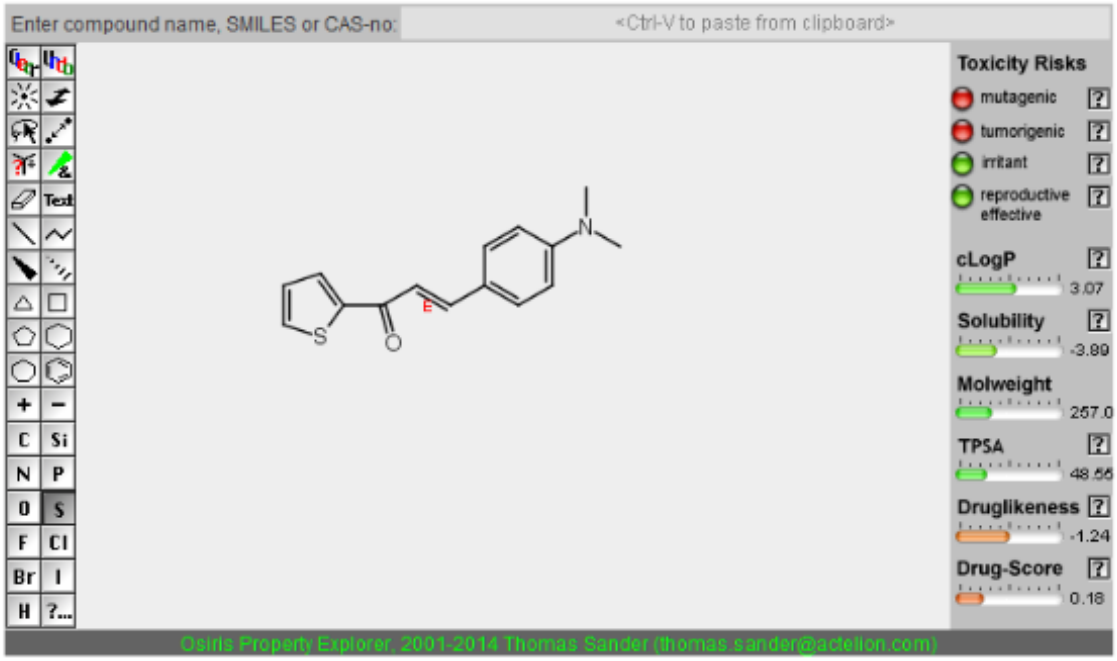
IN-SILICO TOXICITY PREDICTION

All the data set molecules were subjected to the toxicity risk assessment by using Osiris program, which is available online. The OSIRIS property Explorer shown in this page is an **integral** part of Actelion's in house substance registration system. It allows drawing chemical structures and also calculates various drug relevant properties whenever a structure is valid. Prediction results are color coded in which the red color shows high risks with undesired effects like mutagenicity or a poor intestinal absorption and green color indicates drug-conform behavior.

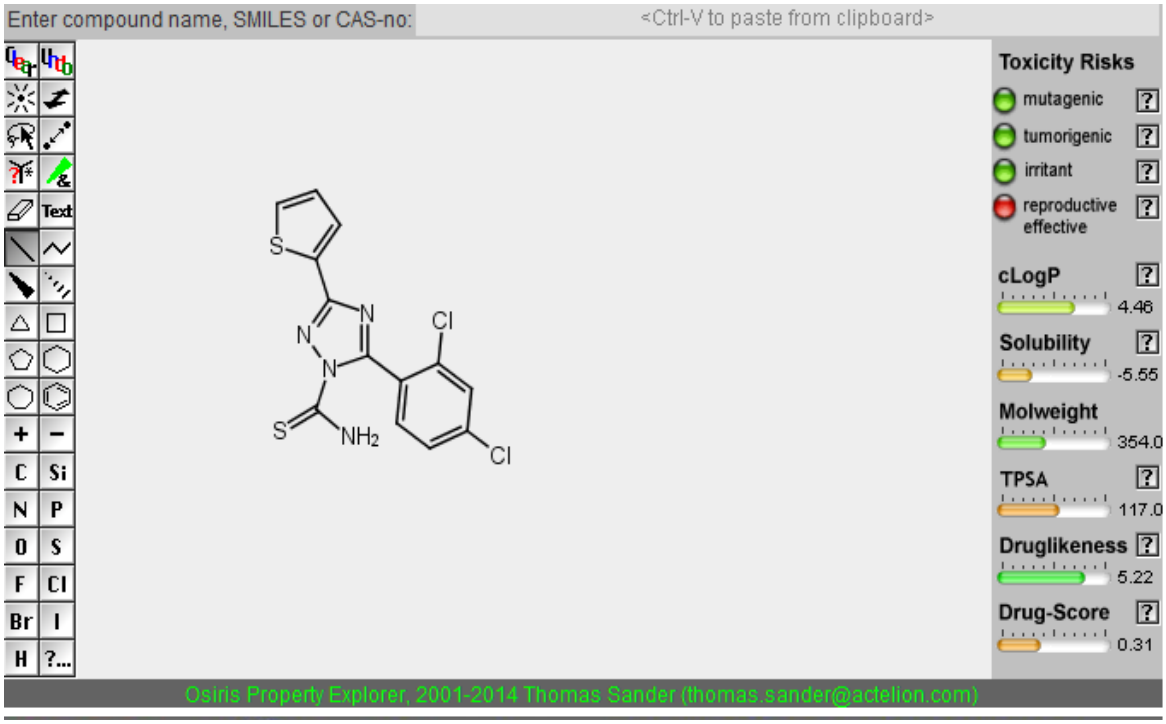
Table no: 8

SAMPLE	DKS1	DKS2-1	DKS3
MUTAGENIC	-	+	+
TUMORIGENIC	-	+	+
IRRITANT	+	+	+
REPRODUCTIVE EFFECTIVE	+	-	+

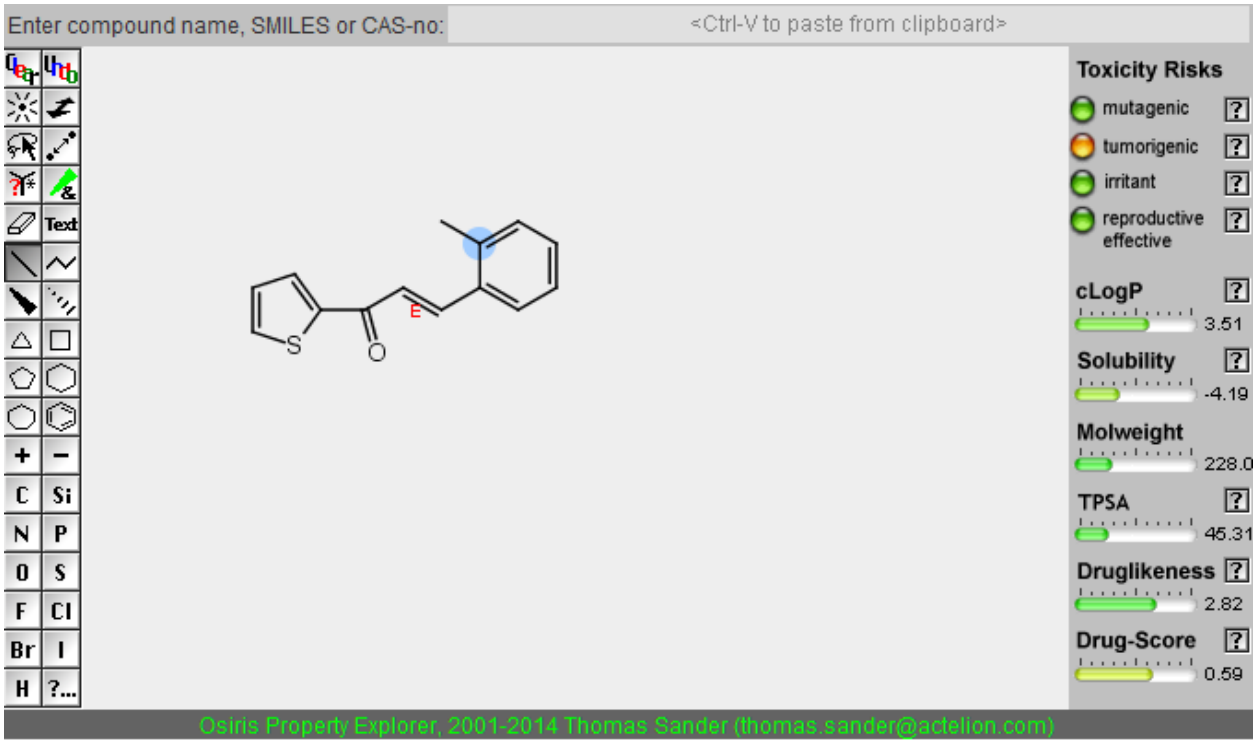
SAMPLE CODE : DKS1



SAMPLE CODE : DKS2-1



SAMPLE CODE :DKS3



BIOLOGICAL EVALUATION

The final pure compounds were screened for Anti-mycrobial activity by in vitro method called Microplate Alamar Blue Assay (MABA)

Table no: 9

S.NO	SAMPLE CODE	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	3.12µg/ml	1.6µg/ml	0.8µg/ml	50µg/ml
1	DK S1	S	S	R	R	R	R	R	R	R
2	DKS 2-1	S	S	R	R	R	R	R	R	R
3	DK S3	S	S	R	R	R	R	R	R	R

NOTE:

S- Sensitive

R-Resistant

Strain used:M.Tuberculosis(H37RV strain)

Here are the standard values for the anti –TB test which was performed.

Pyrazinamide-3.125µg/ml

Streptomycin-6.25µg/ml

Ciprofloxazin-3.125µg/ml

NOTE:

S- Sensitive

R-Resistant

Strain used:M.Tuberculosis(H37RV strain)

Here are the standard values for the anti –TB test which was performed.

Pyrazinamide-3.125µg/ml

Streptomycin-6.25µg/ml

Ciprofloxazin-3.125µg/ml

Table no: 10

SAMPLE DRUG PHOTOGRAPH

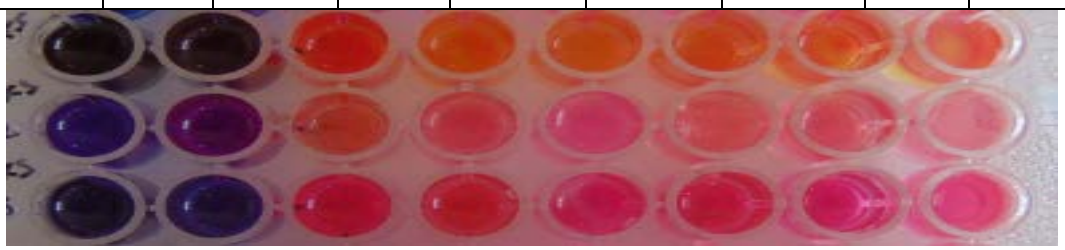
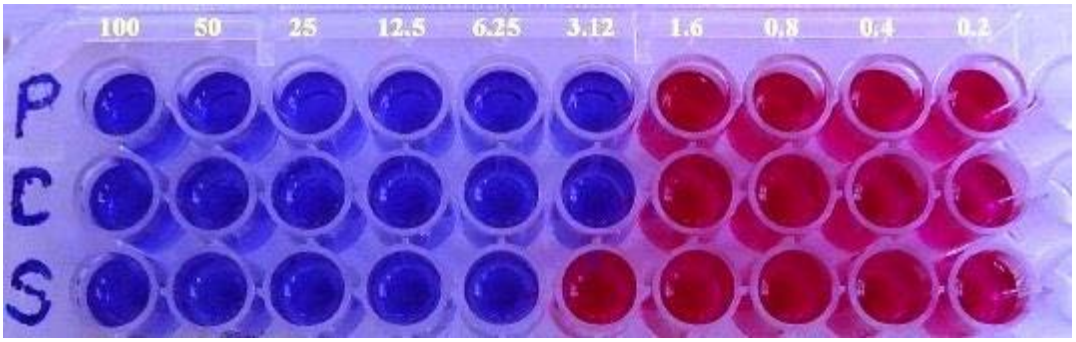
S.NO	SAMPLE CODE	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	3.12µg/ml	1.6µg/ml	0.8µg/ml	50µg/ml
1	DKS1									
2	DKS2-1									
3	DKS3									

Table no: 11

STANDARED DRUG PHOTOGRAPH



DISCUSSION

Finally all the reports were discussed, that the purity of the compounds are confirmed by sharp melting point and TLC. Then the molecular weights of the compounds are confirmed by NMR and the functional groups of the formed structure were confirmed by absorption bands obtained in the spectra. Among 3 compounds, one compound was 100% pure in nature it was concluded by GC-MS.

Toxicity of the compounds are reported, that 2 compounds are non-toxic and one compound was slightly toxic in nature.

The biological activity of the compounds are evaluated by MABA and the final results concluded, that all the 3 compounds are not match with standard drugs. The activity of the compounds sensitive only at 50µg/ml.

6.SUMMARY AND CONCLUSION

- ✓ Methoxy mycolic acid synthase 2 (1TPY) a critical enzyme for the growth of Mycobacterium tuberculosis was chosen for our study after review of literature.
- ✓ A database of 200 scaffolds with high prospect of inhibiting the target 1TPY were carefully chosen by making changes to the known hit molecules , here the chalcones and thiophene nucleus.
- ✓ Candidate molecules were designed and docked against 1TPY protein using Argus lab 4.1 software.
- ✓ Three molecules with good Docking score (lower binding energy) and interactions were shortlisted for synthesis. The reaction conditions were optimized.
- ✓ The selected molecules were subjected to Toxicity Prediction assessment by OSIRIS software. The results are color Coded as green color which confirms the drug likeness
- ✓ The molecules were labeled as DKS1, DKS2-1,DKS3 and synthesized with satisfactory yield.
- ✓ Purity of the synthesized compounds was ensured by repeated recrystallization .The compounds were evaluated by TLC. and Melting point determination.
- ✓ The characterization of the synthesized compounds was done using Infra-red, Nuclear Magnetic Resonance and Mass spectroscopic methods.
- ✓ The final pure compounds were screened for Anti- mycobacterial activity by in vitro method called Microplate Alamar Blue Assay (MABA).

CONCLUSION

- ✓ Our work concludes that our synthesized molecules are effective in inhibiting enzyme Methoxy mycolic acid synthase 2 (1TPY) which is important **for** the growth of Mycobacterium tuberculosis.
- ✓ All the three compounds gave Docking score between -7.00 to -10 kcal/mol. Pyrazinamide gave Docking score of -5.6 for 1TPY, Streptomycin gave Docking score of -7.4 for 1TPY and Ciprofloxacin gave Docking score of -5.9 for 1TPY. There is correlation between the core and activities of all the three compounds which were tested and compared with the standard drugs. This goes to prove that Methoxy Mycolic Acid Synthase2 (1TPY) is a critical enzyme for anti-mycobacterial activity.
- ✓ The minimum inhibitory concentration of 3 of the synthesized compounds ranged from 50µg/ml which is compared to that of the known anti-TB agents.

  Pyrazinamide - 3.125µg/ml,

  Ciprofloxacin - 3.125µg/ml and

  Streptomycin - 6.25µg/ml.
- ✓ A further refinement to the structure of the synthesized compounds is expected to yield new outlook to the development of promising molecules against the pathogen Mycobacterium tuberculosis.

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